GEORGE R MINOT SYMPOSIUM ON HEMATOLOGY

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WILLIAM DAMESHEK, M.D., AND
F. H. L. TAYLOR, PH.D.
EDITORS

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A NOTE BY THE COMMITTEE ON THE GEORGE R MINOT ANNIVERSARY VOLUME

Following a testimonial dinner on the occasion of the sixtieth birthday of Dr George R Minot in 1945 a group of his colleagues conceived the idea of the preparation of an Anniversary Volume of manuscripts to be published in honor of Doctor Minot by his colleagues co workers and former associates

A Committee was formed composed of W B Castle Boston W Dameshek Boston (Chairman) R L Haden Cleveland E B Krumbhaar Philadelphia E Meulengracht Copenhagen Denmark O H P Pepper, Philadelphia R M Suarez San Juan Puerto Rico F H L Taylor Boston (Secretary) G H Whipple Rochester N Y L J Witts Oxford England Invitations to contribute articles were extended to a world wide group of active workers in the field of the blood and to others who had in the past been closely associated with Dr Minot

It appeared eminently desirable that the Anniversary Volume should have full publication in the literature and the Publishers and Editorial Board of BLOOD *the Journal of Hematology* graciously opened their pages for the receipt of these manu scripts

The publication of the Anniversary Volume has been made possible by generous gifts from the Lederle Laboratories Inc of Pearl River N Y and Mr J K Lilly of Eli Lilly and Company Indianapolis Indiana We especially wish to thank Mr Henry M Stratton our publisher for his active interest and guidance and for volunteering to underwrite personally the production of the series of articles and of the bound volumes to follow Our thanks are also due to Dr Henry J Tagnon of the Memorial Hospital New York for his aid in the translation of some of the manuscripts

The members of the George R Minot Anniversary Volume Committee deem it a distinct pleasure and a great honor to dedicate these articles by his colleagues and friends to Dr George Richards Minot

A NOTE BY THE EDITORS

The publication of these eighty odd articles in honor of Dr George R Minot has taken place in regular issues of BLOOD *The Journal of Hematology* beginning with the January 1948 issue and continuing in more or less alternate issues through February 1949 The various articles have been assembled as far as possible in groups of related subjects beginning with that of pernicious anemia whose treat ment brought Dr Minot his greatest renown

The Editors have taken the liberty of making notations and minor changes in certain manuscripts always for the sake of greater clarity Editorial footnotes are suitably marked such notes repre sent the opinions of the Editors and not necessarily those of the authors of the papers We apologize in advance for any errors which may have crept in they are unintentional

WILLIAM DAMESHEK
F H LASKEY TAYLOR

FOREWORD

This is a foreword to the series of articles written by his friends and his co-workers to celebrate the achievements of George Richards Minot It is a good thing thus to give recognition to high scientific accomplishment it warms the heart of both the donor and the recipient of the recognition and it reminds other workers that creative thinking is regarded as worthy of praise

The history and the specific contributions of George Minot are recorded in the bibliography and curriculum vitae which appear on the following pages Perhaps it is pertinent to inquire what qualities may be associated with this kind of accomplishment

First of all George Minot is George Minot and no one in the world is quite like him Of course like almost everyone to whom a Festschrift is dedicated he has been industrious and persistent He had been surrounded by tradition all his life and in some ways his reactions and his thinking are highly traditional but this habit of mind is combined in an extraordinary linkage with an insatiable curiosity and an avidity for understanding that has driven his mind into new and startling regions of thought That curiosity is a major motive for George Minot is attested by his own writings To solve human problems an active creative imagination and scientific curiosity are necessary tools

Indeed the direct desire to explain something otherwise inexplicable may be said to be one of the best motives for research Its validity in the case of George Minot is shown not only by his own classic discovery of the effectiveness of an adequate dosage of liver in pernicious anaemia but by his capacity to stimulate curiosity in others and to gather around himself young men who have curiosity similar to his The articles to which this note is a foreword make up an adequate example of this side of his character

To the desire to learn and the desire to encourage curiosity on the part of his students is to be added a great desire to be useful to the sick If George Minot is to be judged by his motives—a zeal for knowledge an enthusiasm for teaching and a humane urge to alleviate suffering and disability—these motives make up a list that lesser personalities may envy

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George R. Minot

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Born in Boston Massachusetts December 2 1885 Son of James Jackson and Elizabeth (Whitney) Minot

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Assistant in Medicine	Massachusetts General Hospital	1915–18
Assistant in Medicine	Harvard Medical School	1915–18
Visiting Physician	St Luke s Convalescent Home	1916–18
Assistant Consulting Physician	Collis P Huntington Memorial Hospital	1917–19
Associate in Medicine	Massachusetts General Hospital	1918–23
Physician	Collis P Huntington Memorial Hospital	1919–23
Assistant Professor of Medicine	Harvard Medical School	1918–27
Consulting Physician	Massachusetts Charitable Eye and Ear Infirmary	1922–24
Chief of Medical Service	Collis P Huntington Memorial Hospital	19 3–28
Physician to Special Clinic	Massachusetts General Hospital	19 3–25
Associate in Medicine	Peter Bent Brigham Hospital	1925–28
Special Consultant in Diseases of the Blood	Massachusetts General Hospital	1925–27
Member Board of Consultation	Massachusetts General Hospital	1927–
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Chief 4th Medical Service	Boston City Hospital	1928–30
Visiting Physician	Boston City Hospital	1928–48
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Consulting Physician	Beth Israel Hospital	19 9–
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Honorary Fellow New York Academy of Medicine	1933
Honorary Fellow Institute of Medicine of Chicago	1933
Honorary Fellow Royal Society of Medicine London	1932
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Honorary Member Kaiserlich Leopold Caroline Deutsche Akademie der Naturforscher (Halle)	1935
Honorary Member Society Biological Chemists (India)	1936
Honorary Member Finnish Society of Internal Medicine (Helsingfors)	1938
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Member of American Academy of Arts and Sciences	1927
Member of American Clinical and Climatological Association	1923
President	1932
Member of National Academy of Sciences	1937
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Alpha Omega Alpha	1911
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Advisory Council of Physicians Forum	1946
President Senior Staff Boston City Hospital	1947

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Cameron Prize University of Edinburgh	1930
Gold medal National Institute of Social Sciences	1930
Gold medal and Award Popular Science Monthly	1930
Moxon medal Royal College of Physicians London	1933
John Scott Medal of City of Philadelphia	1933
Gold Medal of Humane Society of Massachusetts	1935
Nobel Prize in Physiology and Medicine jointly with William P Murphy and George H Whipple for work on liver treatment of the anemias	1934
Scroll Award of Associated Grocery Manufacturers of America	1936
Gordon Wilson Lecturer and Medalist American Clinical and Climatological Association	1939
Distinguished Service Award American Medical Association	1945

PART I

PERNICIOUS ANEMIA

TREATMENT OF PERNICIOUS ANEMIA BY A SPECIAL DIET*

By George R Minot M D, and William P Murphy M D

THIS PAPER concerns the treatment in a series of forty five cases of pernicious anemia in which the patients were given a special form of diet While the problem of diet in the treatment of pernicious anemia is by no means new in our opinion its possible importance has not heretofore been generally recognized In 1863 seven years after the publication of Addison s second but best known description of the disease now called pernicious anemia Habershon[1] wrote concerning this condition

Many patients at an early stage completely recover under the influence of bracing air and a nutrient and stimulating diet Other early investigators of the disease as Biermer[2] in 1872 and Pepper[3] in 1875 appreciated the desirability of prescribing easily digested foods as a form of medication but no greater emphasis was placed on the value of diet Osler[4] however in 1885 mentioned that cases [of pernicious anemia] appear to have got well with change of air and a better diet after resisting all ordinary means

During the last half century many clinicians following the suggestions of the pioneer writers on the subject of pernicious anemia have advised various kinds of diet as an aid to induce a remission of the disease More often than not the recommendations have been of a general sort as might be given for many persons with an impaired condition of the gastro-intestinal tract which always is present in pernicious anemia Thus food for the pernicious anemia patient often has been selected because it appeared to be easily digested or because it seemed particularly nutritious and strength giving Rarely diets have been chosen for some assumed direct effect on the blood

The constant presence of achylia gastrica in pernicious anemia and the frequency of an abnormal bacterial activity within the intestines have been two main reasons for establishing certain forms of dietotherapy in the disease On these accounts Fenwick[5] in 1880 and Naegeli[6] among others recommended diets relatively sparing in farinaceous foods and relatively rich in protein For similar reasons yet in contrast to the majority Hunter[7] in 1890 and others have advised quite the opposite type of diet Grawitz[8] recommended a diet composed chiefly of fresh vegetables followed by one with generous amounts of protein The idea that forced feeding with any sort of food but especially meats is valuable to make weak and feeble individuals healthy and strong has caused the frequent use of this form of

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Meats and green vegetables, partly because of their iron content have for a long time been thought to be useful to improve an anemic state of the blood Meat apparently has been chosen at times simply because it contained blood which was supposed to be beneficial as food for persons who had an insufficient blood supply The scientific foundations of the value of iron containing foods to affect the blood forming organs were laid by Menghini[10] in 1746 when he showed that iron could be increased in the blood by feeding such foods to animals About 200 years later Gibson and Howard[11] made important observations on the effect of a high iron content of the diet in anemia and showed that in pernicious anemia it can have a most favorable influence on iron metabolism They also showed that in cases con stantly losing nitrogen, a positive nitrogen balance could be obtained without forced feeding

One thus finds that the diet usually advised for the pernicious anemia patient is one containing a relatively high nitrogen content and often a relatively large number of calories The recommendations of Smith[12] and of Barker and Sprunt[13] are of this sort and like some others the latter wisely recommend that the food be selected with a view to giving an ordinary well balanced diet to replace a quanti tatively deficient and qualitatively ill balanced one, on which these patients are apt to have placed themselves during their illness

In spite of attention to diet for the anemic patient, the influence of food on blood formation and destruction has received comparatively little consideration and special sorts of food because of some particular effect have seldom been chosen for patients with pernicious anemia

Complete starvation in man is not considered to cause anemia but may do so in animals However it is known that improper food can cause and suitable food alleviate anemia for example, the iron starvation anemia arising in infants who have partaken too long of only a milk diet and who can be cured by food particu larly containing complete proteins and iron Incomplete diets particularly those low in protein and relatively rich in concentrated carbohydrate food can lead to anemia [14] and even Shakespeare[15] recognized that improper food might impair the state of the blood Likewise patients with conditions due to or associated with vitamin deficiency experience anemia and Jencks[16] has noticed that an abundance of vitamins favors blood regeneration Certain foods including liver may benefit patients with sprue This disease is considered by some partly dependent on a faulty diet and resembles in numerous ways pernicious anemia including the fact that the blood picture in the two diseases may be quite similar Carnivorous animals and thin persons tend to have a greater percentage of hemoglobin in their blood than herbivorous animals and fat persons [17] This further suggests as do the obser vations of Morawitz and Kuhl[18] on man the favorable role that animal protein food may play in blood formation although dehydration may account for the differences observed

Some of the earlier experimental work concerning the effect of food on blood

regeneration is reviewed by Pearce, Krumbhaar and Frazier [19] Adequate proteins as well as iron are necessary for the formation of hemoglobin Certain proteins will not suffice such as gliadin [20] However, the amino-acid tryptophane may have a special ability to enhance blood formation [21] The most important recent work concerning the effect of food on blood regeneration has been done by Whipple and Robscheit Robbins and their associates [2] Their carefully controlled work on dogs has demonstrated clearly the value of certain foods especially liver on accelerating blood regeneration following acute hemorrhage and the value of iron added to the diet to decrease the anemia due to chronic blood loss

McCollum[22] has pointed out that liver and kidneys give an exceptionally high quality protein for a low protein intake and can enhance remarkably the growth of animals These foods are rich in nucleins and Calkins Bullock and Rohdenburg [4] have shown that the products of nuclein hydrolysis can stimulate growth Whipple[23] has suggested that in pernicious anemia there may be a scarcity of the material from which the stroma of the red blood cells are formed or that a disease of the stroma forming cells of the marrow exists Thus theoretically perhaps liver and other foods rich in complete proteins may enhance the formation of red blood cells in this disease especially by supplying material to build their stroma

Fresh red marrow was first used as a means of treatment for pernicious anemia by Fraser[24] in 1894 He reported beneficial results when a patient ate for some time about 100 Gm a day It was then and has since been given apparently on the supposition of some hormone effect Thus numerous reports have appeared concerning the use of preparations of small amounts of concentrated bone marrow but without definite evidence of advantage to the pernicious anemia patient Reports regarding the effect of eating generously of fresh marrow are few and brief but suggest that it may be beneficial The nutritional composition of red bone marrow is similar to that of liver and kidneys If generous amounts of red marrow and liver can improve the state of the blood in pernicious anemia may their influence not be due to the same but unknown cause?

Various investigators have commented on the blood destroying properties of certain substances derived from fats and the rôle they may play in pernicious anemia Stoeltzner [7] recently has reviewed the subject Also lipoids have been shown by Baker and Carrel[25] to be a factor in serum that can inhibit growth Thus founded on somewhat theoretical grounds it seemed to us as it did to Stoeltzner [7] and to Gibson and Howard [11] that decreasing the amount of fat in the diet of the pernicious anemia patient might have a favorable effect on the state of his blood Excess of fat in a diet is considered by some to favor putrefaction within the intestine a condition frequent in pernicious anemia Hence one might attribute any benefit derived from a low fat content of the diet to alterations in the bacterial flora rather than to some more direct effect on blood formation or destruction

A further hypothetic reason for decreasing the fat in the diet is that we have noted it is not uncommon for these patients to have consumed throughout life unusually large amounts of food rich in fats Patients with pernicious anemia also may give a history of partaking for years of some other type of one sided diet It is common for them to do so after the definite onset of their illness when it is not

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it to date (except temporarily omitted by three) or from six weeks to two and a half years

The special diet[32] used was made as palatable as possible and for each day was practically as follows

1 From 120 to 240 Gm and even sometimes more of cooked calf s or beef liver An equal quantity of lamb s kidneys was substituted occasionally

2 One hundred and twenty grams or more of beef or mutton muscle meat

3 Not less than 300 Gm of vegetables containing from 1 to 10 per cent of carbohydrate especially lettuce and spinach

4 From 250 to 500 Gm of fruit especially peaches apricots strawberries pineapple oranges and grapefruit

5 About 40 Gm of fat derived from butter and cream allowed in order to make the food attractive However animal fats and oils were excluded so far as possible

6 If desired an egg and 240 Gm of milk

- In addition to the above m ntioned foods breads especially dry and crusty potato and cereals in order to allo a total intake of between 2 000 and 3 000 calories composed usually of about 340 Gm of carbohydrate 135 Gm of protein and not more than 70 Gm of fat Grossly sweet foods were not gi en but sugar was allowed very sparingly

This diet is rich in iron and purine derivatives containing about 0 03 Gm of the former and about 1 Gm of the latter

At the time the diet was advised for many of the patients they were able to take only a small amount of food of any sort Under these circumstances they were en couraged to take as much as possible of liver and fruits and at least some vege tables while other sorts of food were not forced During the first week of the diet the intake was often less than a thousand calories After about this period of time the patients usually felt distinctly better and their appetite began to improve Then the food was increased gradually until the complete diet was taken The patients as a rule did so within two weeks after the diet was begun In fact frequently they soon became ravenously hungry and often anxious to eat more than the custom ary allowance of liver and meat

Twenty four of the forty five patients carried out the regimen by weighing por tions of liver and meat and estimating the amounts of the rest of their food for at least three weeks and often for the first six after commencing the diet The other patients like those after leaving the hospital have taken their diet at home fol lowing out written directions but not weighing any of their food Our data strongly suggest that the patients who commenced treatment in the hospital and those few able to have a trained nurse at home have improved on the average rather faster and to an even b-tter degree than the others When the patients had remained much better for many weeks their diet was sometimes modified particularly by decreasing the amount of liver and fruit

The therapeutic regimen for these forty five patients besides the special diet included rest usually at first in bed for twenty four hours a day All but three also took each day about 15 cc of diluted hydrochloric acid (U S P) These three however improved at least as much as the majority of the others None of the pa tients received any especial treatment shortly before or after the diet was begun except as follows A man aged 69 with pronounced spinal cord lesions and ad

unusual to find that they have a disgust for meat Pernicious anemia is rare in certain parts of the world where diets are quite different (containing fewer dairy products less free sugar and muscle meat) from those of the northern parts of Europe and America in which areas the disease is relatively common These different facts permit one to speculate on the possible partial role that some nutritional excess or deficiency may play in the etiology of the disease Similar thoughts have occurred to others including the idea that a vitamin deficiency might be a causative factor as has been mentioned for example by Elders [29]

Leafy vegetables and fruits usually are considered desirable for anemic patients especially because of their iron content and strawberries rich in iron appear beneficial for patients with sprue a disease as noted resembling pernicious anemia We prefer to add these foods to the pernicious anemia patient s diet not only because they are healthful ones for any person to eat but also because as Whipple and Robscheit Robbins[30] have shown certain ones have an especially favorable influence on hemoglobin production It is quite probable however, that their chief effect is not because of their iron content It seems that such a factor as the character of the proteins or amino-acids in the diet is of much more importance than the iron content for pernicious anemia patients

Numerous authorities hold the view that an intestinal bacterial toxemia plays an important etiologic role in this disease One may choose to believe that any benefit these patients derive soon after beginning to take certain foods is to be attributed to changing rapidly the intestinal flora thus decreasing a bacterial toxemia rather than considering that the foods influence in some unknown but more direct manner the formation or destruction of red blood cells

Gibson and Howard [11] taking cognizance of Whipple and Robscheit Robbins work and the fact that certain lipoid substances could enhance hemolysis fed pernicious anemia patients a relatively low caloric diet (from 1 500 to 1 900) rich in iron [liver (daily) fruits green vegetables egg yolk] and low in fat and adequate in vitamins A somewhat similar diet but containing a less amount of food rich in purines was recommended by Fenlon[31] in 1921 Gibson and Howard [11] besides demonstrating the favorable influence of their diet on nitrogen and iron metabolism in pernicious anemia and some other anemias suggested that it enhanced a *remission in pernicious anemia and urged its use*

Material Studied and Observations

Following the work of Whipple and Robscheit Robbins we made a few observations on patients concerning the influence of a diet containing an abundance of liver and muscle meat on blood regeneration The effect appeared to be quite similar to that which they obtained in dogs These observations together with the information given above led us to investigate the value of a diet with an abundance of food rich in complete proteins and iron—particularly liver—and relatively low in fat as a means of treatment for pernicious anemia

Observations set forth below have been made on forty five patients with typical pernicious anemia first partaking of such a diet when in a relapse and continuing

it to date (except temporarily omitted by three) or from six weeks to two and a half years

The special diet[12] used was made as palatable as possible and for each day was practically as follows

1 From 120 to 240 Gm and even sometimes more of cooked calf s or beef liver An equal quantity of lamb s kidneys was substituted occasionally

2 One hundred and twenty grams or more of beef or mutton muscle meat

3 Not less than 300 Gm of vegetables containing from 1 to 10 per cent of carbohydrate especially lettuce and spinach

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6 If desired an egg and 240 Gm of milk

7 In addition to the above mention d foods breads especially dry and crusty potato and cereals in order to allow a total intake of between 2 000 and 3 000 calories composed usually of about 340 Gm of carbohydrate 135 Gm of protein and not more than 70 Gm of fat Grossly sweet foods were not given but sugar was allowed very sparingly

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vanced arteriosclerosis was given five transfusions of blood within about six weeks while attempts were made to get him to eat Now, three months later, he remains the least well of all forty five, except for one woman who recently has omitted her diet Blood was transfused to three others at about the time they first took the special diet The red blood cell count of none was over 1 400 000 per cubic millimeter four days after transfusion

The forty five patients represent an essentially consecutive series seen in a relapse, and are all that have taken the special diet except one noted below The series is not entirely consecutive because during the time the forty five cases were seen the following additional ones came under observation

1 Four patients who had had their disease a long time were exceedingly sick able to take little or no food and died within a few weeks after they were seen They at no liver or kidneys

2 Five patients consulting us but once and not taking the special diet Letters indicate that three improved somewhat and two did not

3 One patient that was in much better condition soon after taking the diet This patient is not included in the series of forty five because of several unusual complications

Many of the forty five patients had had definite symptoms due to pernicious anemia for more than two years and two of them experienced such symptoms ten years before taking the special diet A number of the cases were observed during a year or more before the diet was begun others for several weeks and some for only a few days Many of the patients had remained in distinctly poor health and were unable to do their usual work for from a few months to more than a year before eating the food especially prescribed During this time many received various forms of therapy without distinct benefit including transfusions of blood

When the special diet was started the forty five patients that have continued to eat this kind of food fell naturally into the three following groups (1) twelve in their first distinct relapse (2) seventeen in their second relapse (3) sixteen having had two or more relapses It is thus evident that all sorts of variations of the disease occurred among the patients and that the series was not composed chiefly of those in their first relapse following which considerable spontaneous improvement is the rule

The condition of all forty five patients became much better rather rapidly soon after commencing the diet All except one who has recently omitted her diet are now at the least in a very fair state of health and if it were not for disorders in some due to spinal cord lesions would have an appearance to a layman of being essentially well However there are only eleven patients who began the diet a year or more ago two of whom have taken it for more than two years Eighteen began taking the diet less than five months ago

One of the earliest signs of improvement has been a change in the frequency of bowel movements believed to be due particularly to the diet and probably not to diluted hydrochloric acid Within a few days those who had had a tendency to diarrhea often began to have one formed stool a day while interestingly enough those who had had normal movements or had been constipated frequently had for several days a few loose stools in each twenty four hours The latter patients then had a more natural regularity of their bowel movements and a more normal stool

than they had had for some time before the diet was taken The laxative effect of the diet has been observed also to occur in some normal persons

Clinical improvement has been obvious usually within two weeks This has been heralded in the peripheral blood before the end of the first week by the beginning of a most definite rise of the reticulocytes (young red blood corpuscles) of from about 1.0 per cent to usually about 8.0 and even to 15.5 per cent of all the red blood cells This rise occurred in all fifteen patients that have had such counts made every day or so for from one to three weeks before and some weeks after beginning the diet By the end of the second week these cells usually had returned close to their normal percentage Later when the red blood cell counts were distinctly high it

Average Red Blood Corpuscle Count

Before diet started		After diet started					
		About 1 month		About 2 months		4 to 6 months	
Number of cases	Average R.B.C. count	Number of cases	Average R.B.C. counts	Number of cases†	Average R.B.C. counts	Number of cases†	Average R.B.C. count
	millions		millions		millions		millions
19	0.90	19	3.28	15	4.08	12	4.50
15	1.60	15	3.25	13	4.09	10	4.54
11	2.30	11	3.83	9	4.41	5	4.47
45	1.47	45	3.40	37	4.16	27	4.50

The figures represent the count per cubic millimeter before and after starting special diet in three groups of cases of pernicious anemia (1) with less than 1.2 million (2) having from 1.2 to 2 million and (3) having from 2 to 2.75 million before diet was begun Also averages for all forty five cases are shown

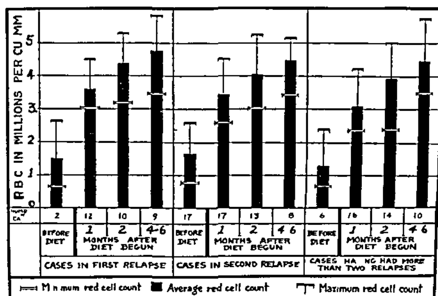
† The differences in the number of cases after about one month is because some have not taken the diet for as long as two and others as long as four months

was *frequent to find* as we have *noted formerly* an *abnormally small number* of reticulocytes Before they began to increase the icterus index of the blood serum in these fifteen patients started to fall and soon the yellow tint of the patient s skin disappeared This index reached normal in from two to four weeks and often has fallen to below normal even when the red blood cell count had increased to only 2 500 000 per cubic millimeter [33]

The accompanying chart and table give in a synopsized manner the trends of the state of the blood in the forty five patients taking into consideration on the one hand the character of the case and on the other the level of the red blood cells when the diet was begun The data are given for all forty five patients before and about one month (from four to six weeks) after the diet was started Although all the patients have been observed repeatedly data can be given for only thirty-seven at the end of about two months (from eight to eleven weeks) of treatment and for twenty-seven between four and six months after treatment began because eight have taken the diet for less than two months and eighteen for less than four months As a measure of the patient s condition we have chosen to give in the table and chart the red blood cell count rather than the hemoglobin percentage partly because the latter in pernicious anemia may be at about the same high level

(80 per cent) with red blood cell counts of from 2 5 to 4 million per cubic milli meter It is recognized that figures for both may vary considerably within a few hours The figure used in synopsizing the data often represents in each instance an average of several counts made within a few days of each other

Inspection of the chart and table shows the rapidity with which the red blood corpuscles increased the high level they attained at the end of about one month, two months and from four to six months after the diet was begun and the rather slight differences that occurred in the bloods in the cases falling into the three groups based on the number of relapses that had occurred The percentage increase of cells (and the same is true of the hemoglobin) at the end of a month was usually very



Red blood cell counts in forty five cases of p rnicious anemia before and after beginning sp cial diet Cases grouped according to the number of relapses the patients had had One and two months after di t began indicates an approximate amount of time and for any given case is not less and often somewhat more than four or eight eeks The differences in the total number of cas s after about one month are caused by the fact that some patients have had the diet for less than two and others for less than four months

much greater in patients starting the diet when their red blood cell count was less than 1 200 000 per cubic millimeter than in those in whom it was distinctly higher This occurs in other pernicious anemia patients rapidly restoring their blood The blood of patients with rather high counts of their red blood corpuscles and prominent signs of injury to the spinal cord responded more slowly perhaps and less well than others and as is to be expected no striking change occurred in very marked symptoms or signs due to spinal cord degeneration

In pernicious anemia remissions after two relapses are frequently less marked than previous ones so that the red blood cell count is apt to be lower in a third or subsequent relapse than in a former one In spite of the excellent remissions our patients had soon after beginning the diet the data in the chart show what might

be expected namely that not only did the third group of patients (those having had more than two relapses) have on the average a slightly lower red blood cell count before the diet was started but also that afterward their counts were apt to increase more slowly and not become quite so high as in the other two groups Only four of the patients had red blood cell counts as low as between 3 and 2 5 million per cubic millimeter after taking the diet for about a month The cases of three belong to this third group Even so two had 4 000 000 or more red blood cells per cubic millimeter at the end of four months The other the patient transfused several times has now after three months of dieting only 2 600 000 per cubic millimeter However his hemoglobin has risen from 25 to 70 per cent The fourth case with a red blood cell count of less than 3 000 000 per cubic millimeter at the end of a month belongs to the second group and now two and a half months after the diet was started shows a red blood cell count of 3 300 000 per cubic millimeter

The data from which the table and chart were prepared have been analyzed in various ways and the following statements indicate in a different manner than they do what satisfactory improvement was shown in the patients blood Seventy six per cent of all the patients had _ 000 000 or less red blood corpuscles per cubic millimeter with their hemoglobin usually 55 per cent or less before beginning the diet In contrast to this approximately a month (from four to six weeks) later 91 per cent had over 3 000 000 and 42 per cent over 3 500 000 red blood cells per cubic millimeter with corresponding rises in the hemoglobin percentage After taking the diet for about two months (from eight to eleven weeks) 89 per cent (of the 37 that had taken the diet this length of time) had 3 500 000 or more red blood corpuscles per cubic millimeter while 73 per cent had 4 000 000 or more All had a hemoglobin of approximately 80 per cent or over None of the patients studied after they had eaten the food selected for them for between four and six months had less than 3 500 000 red blood cells per cubic millimeter 81 per cent had 4 000 000 or more and the counts of 30 per cent were over 5 000 000 per cubic millimeter The hemoglobin was 80 per cent or above in all often 90 per cent and in several cases reached more than 100 per cent However it is to be noted that none of these cases observed between four and six months after the diet was started had appeared as advanced as several of those in patients that improved the least but which have not yet had the diet *for four months* The observations on the eighteen patients who have been on the diet for more than six months show that their count may fluctu ate though it has remained above 3 200 000 per cubic millimeter and usually has been found over 4 000 000 with the hemoglobin remaining 80 per cent or more There are three exceptions to this statement for three patients had a relapse about eight weeks aft r changing their diet One did so a year and another seven months after the special diet was begun Both had for two or three weeks a count slightly below 3 000 000 per cubic millimeter Their red blood cells and hemoglobin then very rapidly increased under rest and on eating an increased amount of liver and fruit The third patient s red cell count was 4 200 000 per cubic millimeter a month before she changed h r diet She has just resumed the special diet and her red cell count is 1 900 000 per cubic millimeter and hemoglobin 50 per cent

Comment

Cases of pernicious anemia undergoing distinct remissions often show rapid and striking improvement such as occurred in almost all our patients A considerable number of them have made such remarks as I feel better than for several years better than for two years and stronger than after the two times my blood went low before Such statements to be sure are made by pernicious anemia patients having remissions that have not taken this diet and there is no case in this series of forty five that cannot be paralleled by a similar one having a so-called spontaneous remission However the records of eleven cases show that the red blood cell count in the remission following the liver diet has remained distinctly higher, not only than in a former remission but also in three cases higher, for at least two months than in their three previous remissions It is thus again pointed out here that it is rather unusual to find the red blood corpuscle count in a late remission distinctly above the level obtained in several earlier ones A few of the patients observed for many months before they took the special diet ate by our advice relatively small amounts of liver two or three times a week together with other food of the sort contained in the special diet Under such a regimen a moderate *degree of improvement occurred in some to be followed later by a relapse of* their case It was rather striking that when the same patients were placed on a diet rich in liver they improved markedly This suggests as do similar observations we made some time ago in other cases that if liver and food like it play a role in improving the blood of p rnicious anemia it is desirable for the patients to take such food daily and in large amounts

The spontaneous remissions of pernicious anemia and the bizarre course it often runs make it notoriously difficult to determine accurately the effect of any procedure on the disease All sorts of therapeutic procedures have been advised many because a few cases improved promptly after their trial Waves of enthusiasm for certain methods have vanished soon when it was shown that the earlier reports of benefit could be attributed readily to the natural course of the disease There is however no doubt as shown by some of the early and more recent investigators that a well balanced nutritious diet sometimes aids to enhance a remission The patient may be helped by numerous other forms of treatment such as those to change the intestinal flora the injection of protein substances the taking of arsenic the transfusion of blood and splenectomy

At least one remission as has been noted by Cabot [34] takes place at some time but at no regular time in about 80 per cent of pernicious anemia cases Precise data are sparse concerning the frequency degree and rate of remissions in similar groups of cases treated in different ways Splenectomy has caused quick and marked improvement in 64 per cent of the patients undergoing this operation while about 15 per cent more have shown some benefit from the procedure [35] However the remissions that followed have been of no longer duration than those heretofore reported as of a spontaneous nature Excluding desperately ill patients Minot and Lee[36] noted in 1917 that about 35 per cent of forty patients treated in no especial *manner* had a moderate or better remission soon after they were seen Following the transfusion

of blood into forty six similar patients about 50 per cent continued to have definitely improved health for at least many weeks than for some time before the procedure Not more than 20 per cent of the ninety-six patients of these two groups soon had rapid and marked increase in their red blood cells An analysis of fifty other cases observed between 1916 and 1923 in sequence except for several in a terminal condition indicates that 45 per cent developed a definite remission soon after we saw them These patients were treated in various ways by numerous physicians but did not eat large amounts of liver or similar food The remissions were seldom of marked degree with the red blood corpuscles reaching 4 000 000 or more per cubic millimeter

No entirely satisfactory data have been found concerning the frequency of remissions following the use of a nutritious high caloric diet and such a regimen as that prescribed by Barker and Sprunt We have treated in this manner twenty five partially selected cases from which it appears that distinct remissions may follow such therapy in about 65 per cent of the instances Even so apparently the red blood cell counts of patients on such a diet and who were improved distinctly at the end of one or two months averaged less than for all forty five who have eaten generously of liver for the same amount of time

The evidence at hand suggests that the dietetic treatment of pernicious anemia is of considerable importance It has been possible to demonstrate in forty five cases seen essentially in sequence that following a diet rich in liver and low in fat a distinct remission of the anemia occurred rather promptly The promptness and rapidity with which the red blood corpuscles and hemoglobin increased coincident with at least rather marked subjective improvement in the sense of well being and clinical appearance of all the patients and the strikingly better health of many is at least unusual in pernicious anemia It is also not customary for the red blood cell counts during remissions of pernicious anemia to be so frequently of the height that occurred in these patients We are inclined to believe that something contained in the foods rich in complete proteins is particularly responsible for the improvement in the state of the blood The low fat content of the diet is assumed to have a less important effect than the character and amount of protein although probably excess of nitrogen per se is unimportant If liver and similar food is of value every means must be taken including the skill of the nurse and cook to get patients to eat daily as much as possible preferably 200 Gm or more Failure could be attributed to taking too little of such food

There are no data to indicate whether the remissions in these forty five cases will last longer than those of others

It is possible that this series of cases eventually may be proved to be unusual in that there happened to be treated a group that would have taken a turn for the better under other circumstances Also time may show that the special diet used or liver and similar food is no more advantageous in the treatment of pernicious anemia than any ordinary nutritious diet Let this be as it may at the present time it seems to us as it has to Gibson and Howard that it is wise to urge pernicious anemia patients to take a diet of the sort described

Summary

The dietetic treatment of pernicious anemia is of more importance than hitherto generally recognized

Forty five patients with pernicious anemia observed essentially in sequence are continuing to take a special diet that they have now been living on for from about six weeks to two years but which was temporarily omitted by three This diet is composed especially of foods rich in complete proteins and iron—particularly liver —and containing an abundance of fruits and fresh vegetables and relatively low in fat

Following the diet all the patients showed a prompt rapid and distinct remission of their anemia coincident with at least rather marked symptomatic improvement except for pronounced disorders due to spinal cord degeneration Improvement was often striking so that where the red blood cell count averaged for all before starting the diet 1 470 000 per cubic millimeter one month afterward it averaged 3 400 000 and for the twenty seven cases observed from four to six months after the diet was begun the average count was 4 500 000 per cubic millimeter

Patients having had two or more relapses showed on the average slightly lower red blood corpuscle counts about one and two months after commencing the diet than did those who had started it in their first or second relapse

Change in the frequency of bowel movements temporary increase of reticulocytes in the peripheral blood and decrease of the icterus index of the blood serum were among the earliest signs that heralded the patient s better health

All the patients have remained to date in a good state of health except three who discontinued the diet two rapidly improved on resuming it and the other has just commenced it again As the diet was advised for most of the patients less than eight *months ago enough time has not yet elapsed to determine whether or not the re*missions will last any longer than in other cases

Subsequent Observations

Since the data presented in this paper were compiled the following additional information has been obtained The eight patients who had taken the diet for only about one month had red blood cell counts at the end of about two months of between 3 500 000 and 6 000 000 with an average of 4 400 000 per cubic millimeter One of these had but 2 500 000 at the end of one month and now at the end of three and a half months has 4 500 000 per cubic millimeter

The ten patients recorded as having taken the diet for only about two months showed in four to six months after starting it as follows Seven had an average red blood cell count of 5 100 000 per cubic millimeter One who had had about 5 000 000 had but 3 500 000 per cubic millimeter Another who had 2 500 000 per cubic millimeter at the end of the second month had the same number two months later although symptomatically he seemed better The tenth patient could not obtain the proper diet between the second and fourth month and had at the latter time 3 000 000 per cubic millimeter

The patients who had the diet from four to six months or longer when the data were compiled continued in the next two and a half months to have on the average as satisfactory counts except as noted below The majority of these have shown higher counts than formerly Two of the cases have had at three different times red blood cell counts of 6 000 000 or more per cubic millimeter Two patients who have had the diet for more than six months have recently eaten very little liver and their counts have fallen in two months from about 4 000 000 to about 3 000 000 per cubic millimeter The red blood corpuscles of the patient referred to on page 16 as in a relapse increased 3 000 000 per cubic millimeter during the first eight weeks after the diet was resumed

Information at hand suggests that some cases in which transfusion is done many times before the diet is started may respond but little to it

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PERNICIOUS ANEMIA FROM ADDISON TO FOLIC ACID*

By RUSSELL L HADEN M D

A CONSTANTLY fatal disease unexplained at autopsy is always intriguing The mysterious nature of pernicious anemia thus interested Thomas Addison[1] when he described the first group of patients in 1849 He said this is a remarkable form of anemia which has not attracted the attention it really deserves The anemia was profound and of unknown origin The patient became progressively weaker with little wasting and finally died without response to any treatment A postmortem examination did not aid in explaining the problem No real progress was made in solving the puzzle until the discovery of the beneficial effect of liver feeding in 1926 by Minot and Murphy[2] completely altered the outlook of the patient Further research is slowly unraveling the mystery Clinicians still think however of pernicious anemia as a remarkable form of anemia

It is my purpose to discuss historical highlights of this interesting disease from the time of Addison to the discovery of folic acid and to emphasize some important clinical aspects

Idiopathic pernicious anemia is a disease of nutrition characterized by macrocytic anemia histamine refractory achlorhydria combined sclerosis of the spinal cord and a specific response to liver and liver substitutes The anemia alone may be completely relieved by a single chemical compound pteroylglutamic acid (folic acid) The clinical picture is variable the anemia may be minimal only about three fourths of the patients have signs of a cord lesion initially a loss of vibratory sense is usually the earliest and often the only evidence of neurologic involvement achlorhydria is a constant finding

It is a disease of older people In 427 patients studied at the Cleveland Clinic only 5 were less than 30 years of age In a total number of 579 I have seen the anemia began in only 1 individual less than 20 years of age Fifty two per cent of the patients were between 40 and 60 A very large proportion were over 60 when the diagnosis was made

Numerous clinicians beginning with Combe in 1822 [3] reported fatal unexplained cases of anemia which we now recognize as pernicious anemia Thomas Addison however first in 1849 and again in 1855[4] described it as a clinical entity

Why was the disease so called? Addison in his original description speaks of it as a remarkable form of anemia Its approach is first indicated by a certain amount of languor and restlessness to which presently succeed a manifest paleness of the countenance The symptoms go on increasing the patient experiences a distressing and increasing sense of helplessness and faintness he dies either from sheer exhaustion or death is preceded by signs of passive effusion or cerebral oppression All patients in this group were not suffering from true pernicious anemia since 2 recovered and in 3 disease of the adrenal was found at autopsy Addison said in 1855 that he was trying to throw additional light on this

From the Cleveland Clinic Cleveland Ohio

Peter T Bohan lecture given at the University of Kansas Medical School March 1947

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Pernicious anemia is defined as a macrocytic anemia—the red cells are charac teristically large Eichhorst mentions macrocytosis but reports no measurements or even counts in his own cases He does say the number of red cells was about one tenth or one-twenty fifth of normal The first blood count in a patient with p rnicious anemia seems to have been done by Sørensen[13] in 1874 when he counted the blood with Malassez s apparatus and found only 470 000 red cells Sørensen also emphasized the large size of the cells The diameter of red cells had been measured from the time of Leeuwenhoek [14] A monograph on the dimensions of red blood corpuscles by Manassein[15] had appeared in 1872 Eichhorst concluded that the diameter of the cells is almost always increased [16] Laache in his book on the anemias[17] published in 1883 has a long discussion of pernicious anemia and emphasizes the large size of the red cells and the increased color index The de crease in number of red cells the increase in size and the increase in hemoglobin content were thus established very early as characteristic findings

Earlier workers used the red cell diameter as a measure of size With the development of the hematocrit the cell volume was found increased also and a more sensi tive indicator of macrocytosis Capps [18] in his work on volume index found this always increased in pernicious anemia In our series of 579 patients all showed a macrocytosis if untreated except in the rare instance with a coincident iron de ficiency Other clinical conditions will also produce a macrocytosis but seldom so marked as in a pernicious anemia Examples are liver disease intestinal obstruction and nutritional deficiency such as sprue Ehrlich considered the presence of megaloblasts in the p-ripheral blood as diagnostic of pernicious anemia He insisted that these were pathologic nucleated red cells and not simply very young cells Al though Ehrlich and others believed that the diagnosis of pernicious anemia should not be made without the finding of m-galoblasts in the blood this view is no longer held A diagnosis should never be made of untreated idiopathic pernicious anemia in the absence of a macrocytosis of the red cells

The presence of an achlorhydria refractory to histamine stimulation is an es sential finding All clinicians now accept the fact that idiopathic p rnicious anemia should never be diagnosed if free hydrochloric acid be present on gastric analysis The achlorhydria has been a most important factor in the final solution of the origin of the disease Addison and Biermer knew nothing about achlorhydria

How did it become recognized that this was a necessary part of the symptom complex? Test meals were not done until relatively late in clinical medicine Cahn and von Mering[19] first studied the acid in healthy and diseased stomachs in 1886 During the next ten years many articles on the subject appeared in England on the continent and in this country It was soon noted by numerous investigators that when no free hydrochloric acid was found the patients were frequently anemic and that the anemia belonged in the group already designated as pernicious As late as 1900 however Faber and Bloch[20] could collect only 33 cases of pernicious ane mia on whom a test meal had been done Martius and von Lubarsch [21] in the first monograph on achylia gastrica in 1897 reported both pernicious anemia and

condition when he discovered the disease of the adrenal glands known as Addison s disease He again emphasized that there was no discoverable cause whatever

Addison recognized the anemia only by the pallor of the skin and the thinness of the blood In 1849 no blood cell counts or hemoglobin estimations had been done Vierordt[5] did the first red cell count in 1851 Funke[6] discovered hemoglobin the same year, and Welcher published the first extensive clinical article reporting blood counts and hemoglobin estimations in numerous diseases in 1854 Thus accurate measurements of the blood came after Addison s *original communication*

Addison s observations made little impression even in England and little more was heard of this remarkable anemia until it was reported independently by Biermer[8] in Switzerland in 1871 At a meeting of the Medical Society in Zurich November 6 1871 Biermer under the title of Progressive Pernicious Anemia described 15 cases of severe anemia He used the name only in a symptomatic sense grouping together anemias of widely different etiology He had previously[9] mentioned similar cases in which he emphasized fatty degeneration of the heart and vessels Biermer stressed the role of pregnancy So some cases he described were evidently what we know now as the anemia of pregnancy His group has been described as a provisional shelter for a multitude of cases [10] He did not think of pernicious anemia as a single disease He again emphasized the finding at autopsy of fatty degeneration of the heart muscle and small vessels

Biermer s report was published in 1872 in the proceedings of the Medical Society in Zurich For some unknown reason it quickly excited the interest of clinicians everywhere Articles on progressive pernicious anemia began to appear rapidly In England Addison s original description was not recalled until stimulated by Biermer s work In 1875 William Pepper[11] in Philadelphia wrote an extensive article of 26 pages on the disease in the *American Journal of Medical Sciences* Pepper says My present purpose is to offer a contribution to this important study by calling attention to a peculiar form of anemia of obscure and fatal character which has recently been redescribed (i e by Biermer) as though it were a new affection under the name of Progressive Pernicious Anemia He then emphasizes that Addison had previously described the disease as idiopathic anemia Pepper s main contribution is his discovery of the extreme hyperplasia of the marrow He considered pernicious anemia as a primary disease of the bone marrow

Many papers on the subject were published between 1875 and 1878 In 1878 Eichhorst s extensive monograph[1] of 375 pages entitled Progressive Pernicious Anemia appeared All cases previously reported were reviewed Eichhorst mentioned Addison s work but gave him little credit Addison he said considered the anemia due to fatty degeneration of the internal organs while we now know that the anemia is primary and the fatty degeneration is secondary Eichhorst described as pernicious anemia cases of anemia which we now exclude The term designated only a group of fatal anemias and included such conditions as true aplastic anemia leukemia and other bone marrow diseases as well as severe anemias due to infection and toxemia Eichhorst did not have our present concept of pernicious anemia as a single specific entity The name he used however has persisted Interest stimulated by these early papers has never abated

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secondary anemia associated with achlorhydria Achlorhydria was not then considered as a necessary part of the clinical picture The first large group of patients with pernicious anemia on whom test meals had been done were reported by Levine and Ladd[22] in 1921 In 107 patients only 3 were found to have free acid In 2 of these 3 patients the diagnosis was questioned In the light of present day knowledge all would be questioned One for instance had had several operations and a persistent diarrhea following an intestinal resection This patient probably had a symptomless obstruction of the small bowel with a macrocytic anemia Recently Goldhamer [23] in a report on the gastric acidity during remission in pernicious anemia, mentions 1000 patients at the Simpson Memorial Institute as having had a test meal without finding free hydrochloric acid in a single one In our series of 579 patients a test meal was done in 546 Free acid was found but once This patient had a typical clinical and blood picture of pernicious anemia with subacute combined sclerosis A technical error was not excluded The test meal was not repeated because the patient died soon after the original examination No special studies were done to exclude other causes for a macrocytic anemia Recently we have studied 2 patients with a macrocytic anemia and a normal gastric analysis Both were found to have a benign chronic intestinal obstruction These 2 patients also had signs of a subacute combined sclerosis

A possible relation of the stomach to pernicious anemia through impaired nutrition was recognized long before test meals were done Immerman[10] in 1877 described pernicious anemia as a disease of nutrition due to faulty absorption of food Austin Flint[24] in 1860 said Nor is it difficult to see how fatal anemia must follow an amount of degenerative disease reducing the amount of gastric juice so that the assimilation of food is rendered wholly inadequate to the wants of the body The English physician Samuel Fenwick especially emphasized this point of view His book Atrophy of the Stomach [5] was published in 1880 Here he recognized severe anemia as occurring with atrophy of the stomach In Chapter 3 on The Relation of Gastric Atrophy to Other Forms of Idiopathic Anemia he remarked that the cases of atrophy of the stomach with anemia reported by him were identical with those described by Addison as idiopathic or pernicious anemia He quoted Addison s description to emphasize the similarity Fenwick thought however that the anemia was produced by interference with nutrition He pointed out that the digestive powers of the stomach were so impaired that the usual postmortem digestion solution of the gastric mucosa did not even take place unless acid were added *and the gastric contents would not digest egg albumin* These observations of Fenwick are most important in the light of present knowledge of the relation of the stomach to pernicious anemia This atrophic condition of the gastric mucosa in pernicious anemia can now be verified in life by gastroscopy

William Hun r long emphasized the relation of the digestive tract to pernicious anemia He considered the gastric atrophy as resulting from a gastritis due to swallowing bacteria and the characteristic glossitis to be produced by a specific micro-organism He believed that a toxin of bacterial origin in the intestinal tract was absorbed into the portal blood and destroyed red cells

The earlier students of pernicious anemia did not recognize central nervous

system involvement In 1887, Lichtheim[27] described 3 patients with severe anemia and involvement of the central nervous system Lichtenstein [8] in 1884 had previously described cases of pernicious anemia with findings suggesting tabes dorsalis We now think these patients had pernicious anemia with subacute combined sclerosis In 1892 Minnich[29] described 2 patients with pernicious anemia who had serious cord involvement and studied the cord at autopsy He found changes especially in the posterior columns of the spinal cord In this country Dana[30] in 1891 in a discussion of degenerative diseases of the spinal cord described a case with extreme anemia and diarrhea which was evidently pernicious anemia with cord involvement In the same year Putnam[31] described 8 patients with combined sclerosis which we recognize as having pernicious anemia from the characteristic anemia and other symptoms It is interesting that few of the early observers did blood counts on their patients so the anemia was evidently quite extreme to be recognized only by pallor or weakness These observers continually emphasized that the nerve involvement is due to poor nutrition resulting from the anemia After 1890 following such early reports numerous articles appeared describing cord lesions In 1902 McCrae[3] reported 50 patients with pernicious anemia from the Johns Hopkins Hospital and found neurologic manifestations in 20 of these In 1900 Frank Billings[33] took as his subject for the Shattuck Lecture in Boston The Changes in the Spinal Cord and Medulla in Pernicious Anemia He emphasized the now well established relation of diffuse cord degeneration and pernicious anemia He thought the anemia and cord changes resulted from a simple toxin which was probably of intestinal origin His article is illustrated with many sections of spinal cord obtained at autopsy

Russell Batten and Collier[34] in 1900 in discussing subacute combined degeneration of spinal cord described this condition as occurring in patients with severe anemia which was evidently pernicious anemia They thought there was no etiologic relation of the anemia to cord changes In the earlier articles there is necessarily much confusion since the criteria for the diagnosis were not clear Many diagnoses were missed and often cases of severe anemia due to other causes were called pernicious anemia

The central nervous system is affected in 80–85 per cent of cases of true pernicious anemia The most common evidence of cord involvement is a diminution of vibratory sense The cord lesion may be the only significant manifestation of the disease it may be more serious than the anemia There is no parallel between the degree of anemia and involvement of central nervous system Subacute combined sclerosis may arise from other causes The cord lesions usually respond at least partially to liver therapy Sometimes the damage to the central nervous system is beyond repair so neurologic symptoms and signs may persist when the anemia is entirely relieved

The proof of the relation of the stomach to the origin of pernicious anemia is a most important discovery It is easy to see how the stomach was early incriminated since the anemia had been conceived of as a wasting disease due to impaired nutrition This was well expressed by Austin Flint[35] in 1860 as already quoted Immerman s classification of pernicious anemia as a disease of nutrition and Fenwick s

secondary anemia associated with achlorhydria Achlorhydria was not then considered as a necessary part of the clinical picture The first large group of patients with pernicious anemia on whom test meals had been done were reported by Levine and Ladd[22] in 1921 In 107 patients only 3 were found to have free acid In 2 of these 3 patients the diagnosis was questioned In the light of present day knowledge all would be questioned One for instance had had several operations and a persistent diarrhea following an intestinal resection This patient probably had a symptomless obstruction of the small bowel with a macrocytic anemia Recently Goldhamer [3] in a report on the gastric acidity during remission in pernicious anemia, mentions 1000 patients at the Simpson Memorial Institute as having had a test meal without finding free hydrochloric acid in a single one In our series of 579 patients a test meal was done in 546 Free acid was found but once This patient had a typical clinical and blood picture of pernicious anemia with subacute combined sclerosis A technical error was not excluded The test meal was not repeated because the patient died soon after the original examination No special studies were done to exclude other causes for a macrocytic anemia Recently we have studied 2 patients with a macrocytic anemia and a normal gastric analysis Both were found to have a benign chronic intestinal obstruction These 2 patients also had signs of a subacute combined sclerosis

A possible relation of the stomach to pernicious anemia through impaired nutrition was recognized long before test meals were done Immerman[10] in 1877 described pernicious anemia as a disease of nutrition due to faulty absorption of food Austin Flint[24] in 1860 said Nor is it difficult to see how fatal anemia must follow an amount of degenerative disease reducing the amount of gastric juice so that the assimilation of food is rendered wholly inadequate to the wants of the body The English physician Samuel Fenwick especially emphasized this point of view His book Atrophy of the Stomach [5] was published in 1880 Here he recognized severe anemia as occurring with atrophy of the stomach In Chapter 3 on The Relation of Gastric Atrophy to Other Forms of Idiopathic Anemia he remarked that the cases of atrophy of the stomach with anemia reported by him were identical with those described by Addison as idiopathic or pernicious anemia He quoted Addison s description to emphasize the similarity Fenwick thought however that the anemia was produced by interference with nutrition He pointed out that the digestive powers of the stomach were so impaired that the usual postmortem digestion solution of the gastric mucosa did not even take place unless acid were added and the gastric contents would not digest egg albumin These observations of Fenwick are most important in the light of present knowledge of the relation of the stomach to pernicious anemia This atrophic condition of the gastric mucosa in pernicious anemia can now be verified in life by gastroscopy

William Hunter [6] long emphasized the relation of the digestive tract to pernicious anemia He considered the gastric atrophy as resulting from a gastritis due to swallowing bacteria and the characteristic glossitis to be produced by a specific micro-organism He believed that a toxin of bacterial origin in the intestinal tract was absorbed into the portal blood and destroyed red cells

The earlier students of pernicious anemia did not recognize central nervous

treatment had been used prior to 1926—iron hydrochloric acid arsenic trans fusion splenectomy removal of infection special diets and drainage of the in testinal tract At times any method of treatment seemed to produce a remission Sometimes the effect of transfusion was lifesaving by initiating a remission No treatment however could be depended on to stay permanently the course of the anemia It was almost always progressive and usually ended in death from anemia unless some intercurrent fatal disease developed

In 1920 Whipple[40] and his associates had begun a systematic study of the effect of different methods of treatment especially food and drugs on experimental hemorrhagic anemia in the dog They found that the most valuable agent in ameliorating the anemia was whole liver Other foods such as red meat had a similar effect to a varying degree but the effect was not so striking as with liver While Whipple was working with hemorrhagic anemia he emphasized in 1925[41] that even in complex anemia such as pernicious anemia anemia with nephritis and cancer cachexia food factors deserve serious consideration in the clinical management of the blood conditions Whipple did not apply his discoveries to clinical medicine however It remained for Minot and Murphy to find in a routine trial of liver in various types of anemias that the response in pernicious anemia was strikingly different from that in other types of anemia They were helped by the knowledge that the level of reticulocytes is an ideal method of gaging response to treatment Minot and Murphy s discovery was first announced in 1926 and was rapidly verified by clinicians everywhere

Liver and liver extracts affect the stroma of erythrocytes only This verified Whipple s idea expressed in 1922[4] that there is a scarcity of stroma building ma terial in pernicious anemia With adequate liver therapy the blood of a patient about to die rapidly responds and returns completely to normal The glossitis and other gastrointestinal symptoms disappear entirely The neurologic symptoms become improved or do not progress at times they disappear entirely There is nothing more dramatic in medicine than the effect of liver therapy on a patient with pernicious anemia Only the use of sulfa drugs and other antibiotics such as penicillin afford such brilliant results

It was soon found that a liver extract acted just as well as whole liver Extracts have been improved until now these are almost perfect in their action A monthly injection of a potent extract will keep the blood normal and prevent the development of a neurologic lesion An extract of normal gastric mucosa has a similar action as one would expect from Castle s discovery

Many attempts have been made to isolate from liver and liver extract a single specific substance responsible for the beneficial effect While highly concentrated preparations have been made no single substance has been isolated In the mean time a single chemical substance has been found which gives a specific blood re sponse in pernicious anemia and related macrocytic anemias Folic acid a substance found in liver yeast spinach and grasses has proved to be necessary for the growth of certain bacteria and to relieve the anemia developing in certain vitamin deficient diets This substance was found to be effective in macrocytic anemias due to a deficiency such as sprue and other related conditions

work begun in 1871 on gastric atrophy as a cause of anemia, have already been mentioned Henry and Osler[35] in 1886 described a case of pernicious anemia as due to gastric atrophy Numerous other clinicians made similar reports Pepper[11] in his very complete article however lays no emphasis on changes in the stomach Then as gastric analyses were more widely employed came the discovery that patients with pernicious anemia had an achlorhydria and finally the conclusion of all clinicians that achlorhydria is invariable in the idiopathic form of the disease Achlorhydria usually if not always precedes the development of the anemia by many years and persists even in complete remissions Free hydrochloric acid is not only absent in idiopathic pernicious anemia but the amount of gastric secre tion is greatly decreased Askey[36] has recently reviewed 47 cases of pernicious anemia reported as showing free hydrochloric acid on gastric analysis He em phasized that none can be considered true Addisonian pernicious anemia by present day criteria

What is the relation of achlorhydria to the causation of pernicious anemia? We are indebted to Castle[37, 38] for the proof that achylia gastrica is a necessary link in the development of the nutritional deficiency producing the disease He showed that a *patient fails to secrete in the stomach some unknown substance* probably a ferment which acts on the food to produce a substance or substances necessary for the maturation of the red cells in the bone marrow and for the normal metabolism of nervous tissue The proof is simple Ground beef partially digested in the stomach of a normal man with normal gastric secretion when fed to a pa tient with active pernicious anemia initiates a remission and causes active blood formation as shown by a rise in reticulocytes and increase in red cells and hemo globin Similar preparations exposed to digestion in the stomach of a person with pernicious anemia cause no reticulocytosis or erythrocytosis in other patients suffering from pernicious anemia to whom the material is fed Such observations proved that a substance supplied by gastric mucosa is a necessary link in the pro tection against pernicious anemia It was also shown that the achlorhydria by itself is not a factor but the ferment is never absent if free hydrochloric acid is present On the other hand the specific ferment may be present if free hydrochloric acid be absent Castle s work furnished the final proof that pernicious anemia is a deficiency dependent primarily on a gastric defect Many workers such as Austin Flint were right in considering the absence of normal gastric digestion as a cause of anemia though they never thought of such specific action as that demonstrated by Castle Pernicious anemia may follow total gastrectomy Meulengracht[39] thinks Brunner s glands in the duodenum supply the specific ferment also If true this explains normal blood formation after some cases of gastrectomy

Castle s work disproved other theories of pernicious anemia *Gastrointestinal* toxemia infection and other possible causes are no longer mentioned The disease becomes a negative one due to the lack of something and not a hemolytic one due to the action of some positive toxic agent *

The discovery of a specific treatment for pernicious anemia is the most dramatic episode in the long history of this serious disease Many different methods of

* However a hemolytic component cau ation obscure is usually present *Eds*

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Folic acid is a single chemical compound (pteroylglutamic acid) which causes a specific response also in pernicious anemia It matures the megaloblasts of the bone marrow so that the blood returns to normal It probably has little effect on the cord lesion The latest reports indicate a cord lesion may even develop while the anemia is disappearing and the blood count is normal

With folic acid the reticulocyte response is not so pronounced as with a potent liver extract but the full effect is excellent and the blood will return to normal No secondary reticulocytosis occurs in a patient treated with folic acid when liver extract is added The question is still unsettled whether liver extract and folic acid give a better result than liver extract alone Folic acid alone should never be used in the treatment of pernicious anemia since it is not the antineuritic factor It fails to prevent the development or progression of neurologic symptoms indicative of subacute combined sclerosis [43]

The anemia of pernicious anemia is due to the lack of a specific red cell maturating factor necessary for the normal development of the erythrocyte This may well be folic acid since the response of the anemia with adequate amounts of folic acid is complete The relation of liver extract to folic acid is now being investigated Liver extract *contains small amounts of folic acid but not enough to explain its anti* anemic action Liver extract has a widespread effect on the individual needing it possibly through its action on cellular metabolism as shown by the feeling of well being exhibited by a person with pernicious anemia after the treatment for a few days with liver extract The rapid clinical improvement is not due to a relief of the anemia It has been suggested that liver extract restores normal pteroyl glutamic acid metabolism possibly by freeing it from its conjugate form in which it normally occurs in foodstuffs According to this concept folic acid is related to the blood lesion only Further research may well show that other specific substances necessary for normal metabolism of nerve tissue are activated by liver extract Liver extract thus acts as an activator of cellular metabolism[44] rather than as furnishing specific substances preventing or relieving pernicious anemia This work suggests that a complex type of cellular enzyme disturbance exists in pernicious anemia The action of liver principle in restoring normal pteroylglutamic metabolism probably constitutes only one of its therapeutic effects

Summary

I have tried to review and clarify steps leading to our present knowledge of pernicious anemia as a clinical and etiologic entity The early history is most illuminating The development of the present concept of this complicated disease is a triumph of medical research Many great names both in clinical and research fields are associated with the advance in knowledge of pernicious anemia Further research will almost certainly clarify problems still unsolved

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TWENTY YEARS OF LIVER THERAPY

By William P Murphy M D

A PERIOD of slightly more than twenty years has elapsed since the introduction of the use of liver for the treatment of the patient with pernicious anemia It may be of interest and instructive to consider how well the early predictions in regard to the effects of this treatment have been substantiated and to summarize briefly the progress which has been made during this twenty year period

The intensive and controlled study of the use of liver in the treatment of pernicious anemia which demonstrated its efficacy and assured its general acceptance was begun in the spring of 1925 The results of these studies as carried out on 45 patients were published in August of the following year [1] Although diets some including liver had been previously tried it remained for this study to establish liver therapy on a quantitative basis as indicated by the following quotation from this first paper If liver and similar food is of value every means must be taken to get patients to eat daily as much as possible preferably 200 Gm or more Failure could be attributed to taking too little of such food

At the end of another year and after observation of the effect of therapy in 105 patients it was possible to predict with greater confidence Successful therapy of pernicious anemia depends on the treatment being properly carried out for a correctly diagnosed case With these conditions established we believe that essentially all patients with pernicious anemia can be benefited and usually markedly and promptly [2]

Even though the method of treatment has been greatly changed and simplified since the two statements quoted above were written the predicted beneficial effect of liver (or its extracts) has been confirmed and the advice contained in them relative to treatment has been found to be as important now with the simplified methods of treatment as it was when whole liver was used Failure to obtain the best results possible are all too frequently the result of the use of insufficient amounts of anti pernicious anemia substances or to their use only after irreparable damage has been done—too little too late

The stages in the progress of treatment from whole liver to an extract of liver for peroral and finally parenteral administration during this twenty year period are so well known that they need not be here repeated Although several patients have been well maintained for years entirely with the use of whole liver or extracts for peroral use the treatment of choice for the great majority of patients is with liver extracts for parenteral administration These extracts are now fairly well standardized and their potency controlled so that the physician has access to highly potent and refined ones which produce a maximal response with a minimum of inconvenience and discomfort to the patient

The most rapid and in most instances the most satisfactory response to treatment during relapse has been found to follow the injection of large doses of extract

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sufficiently early in the course of their disease and in accordance with the principles demonstrated to be most effective the cause of death would rarely be recorded as that of pernicious anemia The causes of death have changed somewhat in respect to frequency of occurrence as the average age of the patient has increased in consequence of the liver treatment Hypertensive cardiovascular disease and malignancy account for a rather high percentage of deaths as might be expected in a control group of comparable age

The incidence of malignant disease in patients with pernicious anemia is not known In the author s[7] series of 578 cases followed during the first twelve years of liver therapy 29 instances of malignancy involving some part of the body were observed an incidence of 5 per cent During that same period malignancy of the stomach caused death in only 4 cases Two more involving the esophagus were noted During the twenty year period 50 instances of malignant disease have been observed Twenty of these involved the stomach and in all but 1 still living was the cause of death Whether or not this indicates a higher incidence of gastric carcinoma than occurs in a group of patients of comparable age without pernicious anemia has not been determined It is quite likely that this is not the case

The recent synthesis of folic acid[8] and its demonstrated beneficial effect on the blood levels in pernicious anemia[9] has stimulated renewed interest in the therapy of the disease Although this new development may be an important step toward solving several questions concerned with the mode of action of liver or its extracts and although the preliminary reports of its use in the treatment of pernicious anemia are encouraging it must be remembered that its use is still in the experimental stage Many of its possible effects are yet to be determined as for example the amount necessary to initiate a satisfactory remission during relapse the amount needed to maintain over a period of years normal blood levels and whether or not that is possible in all patients Its possible toxic effects are not known and its value in preventing or bringing about improvement of the neural disturbances remains to be seen Evidence has already appeared to indicate that it does not control these and that it is not a complete substitute for liver or its extracts in the management of pernicious anemia It is to be hoped that the medical profession will not be stampeded into the use of folic acid as a substitute for liver substances by glowing reports of its value made particularly in the lay press by irresponsible writers who do not have at heart the best interests of the patient with pernicious anemia

Much more study is needed before folic acid can be accepted as a safe and effective substitute for liver and liver extracts Efforts to side step a definite decision in this regard by combining folic acid with liver extract merely increase the cost of treatment without definitely adding to its effectiveness One may confidently say in the light of our present knowledge that liver will do everything that folic acid will do and more for the patient with pernicious anemia

Finally it may be stated with confidence that results of the use of liver or its extracts in the treatment of pernicious anemia during the twenty years justify the early optimism in regard to its value

Some of the patients included among the first group of 45 treated and more of

supplying a high content of anti pernicious anemia substances as elsewhere outlined [3] [4] Thereafter maintenance treatment must be individualized determined on the basis of its effect on the blood levels and on the neural disturbances if these are present The amount of liver extract necessary to maintain the best possible state of health varies greatly from one patient to another The need for adequate therapy cannot be too greatly stressed In his Nobel lecture made after nearly ten years of experience with liver therapy Minot[5] made the following statement

The grave error in treatment is to prescribe too little liver extract or potent substitute Where there is doubt, more rather than less should be given It is essential that the individual receive into his body indefinitely and with regularity enough potent material for his given case Little need be added to this statement in order to condemn an effort to standardize maintenance therapy It has been demonstrated that the amount of liver extract needed to maintain a satisfactory state of health as determined from observation of a fairly large group of patients[6] is that which supplies 15 units about every three and one half weeks The actual intervals varied however in this group of patients from one to six weeks It is obvious from this that one cannot produce the best results if the same dose of extract is given to all patients at the same interval

The more highly concentrated and refined extracts insure the most satisfactory response with the least inconvenience and expense because of the need for less frequent injections There is no valid argument for the use of so called crude extracts in the treatment of pernicious anemia even though disturbances resulting from sclerosis of the central nervous system are present There is much evidence available to confirm the beneficial effect of the concentrated extracts on all of the disturbances characteristic of pernicious anemia including those due to neural damage No evidence has been presented to show that crude extracts are more beneficial in any respect Furthermore their use necessitates greater frequency of injection and they are often distinctly more irritating with greater discomfort for the patient The crude extracts contain a greater amount of solids than do the refined which are probably inert the content of vitamins and other substances which might add to their value has not been found to be greater and some of those used as crude extracts are merely dilutions of the refined

The superiority of parenteral extracts over other forms of therapy is in part due to the careful follow up control of the patient made possible by his return for injections at regular intervals The pernicious anemia patient is subject to the same weaknesses of the flesh as are we all As his condition improves with peroral therapy there is a great temptation to neglect treatment and the check up visit to his physician the result in too many instances is hematologic and neurologic relapse The relatively frequent visits for injection have improved the physician patient relationship so essential to the most satisfactory control The importance of this is emphasized by Minot [5] also in his Nobel lecture when he cautions The physician however must do more for his patient than prescribe a proper amount of liver stomach or the like he should attend to all aspects of the case and not neglect attention to the individual s problems of thought and action

Were it possible for all patients with pernicious anemia to receive treatment

4

THE RELATION OF THERAPY IN PERNICIOUS ANEMIA TO CHANGES IN THE NERVOUS SYSTEM EARLY AND LATE RESULTS IN A SERIES OF CASES OBSERVED FOR PERIODS OF NOT LESS THAN TEN YEARS AND EARLY RESULTS OF TREATMENT WITH FOLIC ACID

By Frank H Bethell M D and Cyrus C Sturgis M D

THE FREQUENT occurrence of nervous system involvement in pernicious anemia the characteristic localization of the lesions in the spinal cord and the extension of the process before specific treatment became available suggested to early observers a common cause of or a cause and effect relationship between the hematologic and neurologic manifestations of this disease However the absence of combined system degeneration or even peripheral neuropathy in many cases of pernicious anemia and the complete dissociation of the severity of the neurologic and hematologic features are not satisfactorily explained by these concepts With the general acceptance of Castle s hypothesis of a conditioned metabolic deficiency as the basic mechanism responsible for the pernicious anemia syndrome the view was often expressed that the changes in the hematopoietic and nervous systems resulted from distinct deficiencies which in turn probably depended upon a primary defect in gastric function Evidence for the existence of such separate deficiencies was difficult to obtain because of the lack of exact in formation pertaining to any of the metabolic factors involved

When effective treatment of pernicious anemia was introduced by Minot and Murphy the importance of evaluating the new therapy in the control of combined system degeneration was at once recognized Over the past two decades many clinical reports bearing on this problem have been published and an extensive review of the literature is not pertinent to the present communication The diver sity of results and conflict of opinions which characterized the earlier experiences with liver and stomach therapy have been largely explained as due to differences in the conditions of observation and in the amount and potency of the medications employed and the modes of their administration It is now the concensus of most observers that with individualized optimal therapy the progress of disease of the spinal cord can be arrested in every patient with pernicious anemia unless serious complications are present that the probability of improvement of the neurologic status and the extent of the benefit which may be expected are inversely related to the duration of the process at the time of institution of therapy that the period during which improvement may be anticipated is largely limited to the first few months after commencement of intensive treatment

The regular administration by the intramuscular route of potent liver extract in doses adjusted to the needs of the individual controlled by periodic clinical and hematologic evaluation is almost a guarantee against further nervous system

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those who were included in the group of 105 reported the following year are alive and well insofar as their pernicious anemia is concerned Except for the complications which have appeared as the result of increasing age and not related to the anemic state many more of these original groups would now be living Few of those who partook of sufficient liver to bring about a satisfactory remission have died from pernicious anemia either as the direct or indirect cause

Not only have these persons been kept alive but they have been maintained in such good physical condition that it has been possible for them to carry on their normal occupations as housewives merchants teachers lawyers physicians etc The distressing and often incapacitating disturbances resulting from damage to the central nervous system have been controlled or completely avoided

Before the introduction of the liver treatment the number of deaths from pernicious anemia in the United States alone had risen to about 10 000 per year It may therefore, be estimated that 200 000 persons with this disease in this country alone have had their life span increased by at least ten years and that there are 100 000 persons now living with this disease who would not be except for their use of liver or its extracts

In closing this brief resume of the experiences in the treatment of pernicious anemia with liver and its extracts during this first twenty year period one cannot better express the outlook for the future than did Minot[5] in the closing words of his Nobel address It seems to me that one may expect in the future more information to be obtained which directly or indirectly will follow as the result of these observations Thus upon the foundations laid by previous investigators do medical art and science build a structure which will in its turn be the foundation of future knowledge

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extent of the pre-existing disease and so cannot be defined in terms applicable to all patients In general improvement of 3 plus indicates freedom from serious disability with persistence of some paresthesia and with slight to moderate ataxia in patients who had had the more severe forms of neural involvement Much of the improvement of these patients may be attributed to recovery from peripheral neuropathy with education of new muscle groups and adaptation to altered proprioceptive pathways playing important parts However the greatly superior results of therapy in patients often with extensive disease whose neurologic manifestations were of short duration suggests that in the early stages of spinal cord involvement nerve recovery may occur [1]

The demonstration of unequivocal peripheral nerve changes in pernicious anemia [-8] explains the transient and fluctuating paresthesias which occur so frequently in this condition Peripheral neuritis is probably always present during the active periods of combined system disease and it may occur in the absence of convincing evidence of spinal cord involvement Patients whose sole neurologic complaint was variable paresthesia or tenderness in the extremities without demonstrable deep sensory disturbances were not considered to suffer from significant disease of the nervous system Evidence of some degree of cerebral involvement was fairly common among the members of this series Yet the multiplicity of factors which may have contributed to the production of mental changes in these patients including anemia malnutrition and degenerative vascular disease and the difficulties involved in comparative measurements on a group of advancing age render the p ecise consideration of the mental status of doubtful significance in the evaluation of long term therapeutic results

Several types of therapy were employed in the initial management of these patients Some of them were among the first cases of pernicious anemia to receive the benefits of the liver diet A considerable number were seen before parenteral liver extracts became available During the years there has been a tendency to substitute refined and concentrated preparations of liver extract for the cruder less potent parenteral extracts and for oral products Nevertheless a number of patients largely for reasons of personal choice have continued to take oral liver extract or desiccated stomach or the cruder parenteral liver extracts It thus becomes possible to compare the effects of different kinds of therapy with respect both to initial responses and to the neurologic status after a long period of time Moreover the information afforded by this analysis may be used in the evaluation of results obtained with new types of antianemia medication In this connection a small series of patients treated with folic acid will be reported with particular reference to changes in the nervous system

Of the 70 cases under observation for ten years or longer there were 45 males and 25 females giving percentages respectively of 64 3 and 35 7 This is a somewhat higher proportion of males than is found in our entire series of over 1 000 cases of pernicious anemia seen at the Simpson Memorial Institute in which the percentage of males is approximately 55 The average age of the patients at the time of diagnosis of their disease was 53 8 years and the age distribution for the two sexes was approximately the same The youngest member of the series was 35 and the oldest

damage Such an ideal therapeutic regimen however, is not employed in the actual management of many cases of pernicious anemia Particularly is this true of those patients who live at a distance from their physicians those who change physicians and those who are seriously influenced by economic considerations It therefore becomes important to evaluate the results of therapy over a long period of time in a series of cases presenting varied manifestations of the disease and differing with respect to type and amount of therapy received It is well recog nized that patients with previously active neuropathies who discontinue treat ment or those whose therapy becomes so inadequate as to permit development of pronounced anemia almost invariably suffer reactivation of their neurologic process It is also true although less commonly observed that patients without previous evidence of nervous system involvement may acquire neurologic manifest ations after omission or gross inadequacy of anti pernicious anemia therapy It may not be so easy however to demonstrate a close relationship in all cases between the progress of disease of the nervous system and continued or recurrent suboptimal therapy as evidenced by relatively slight disturbances of erythrocyte values

The 70 patients comprising the first series to be reported have all been under observation at the Simpson Memorial Institute for ten years or longer When seen initially they were in hematologic relapse and had received no effective antianemia therapy for at least several months prior to their first examination Fifty eight of the patients were previously undiagnosed and untreated The diagnosis was established by the clinical and hematologic features characteristic of pernicious anemia by the absence of other demonstrable conditions associated with macro cytic anemia by the invariable presence of histamine refractory achlorhydria and by the therapeutic response in every instance to the administration of potent anti pernicious anemia medication The neurologic status was evaluated and the cases were classified on the basis of objective as well as subjective evidence of involve ment of the peripheral nerves and posterior columns or of the posterior and lateral columns of the spinal cord Although the distinction between evidence of peri pheral neuropathy posterior column disease only and combined system degenera tion is useful as an indication of the severity of the process and in the interpretation of therapeutic results such a separation is by no means an exact one The extent of the process in each patient was graded primarily on the basis of peripheral nerve and posterior column involvement as one plus to four plus indicating in order of increasing severity 1 plus diminution of vibratory sense and altered reflexes in the lower extremities with paresthesia but without significant dis ability 2 plus loss of vibratory sense and impairment of sense of motion and position in the distal portions of the lower extremities with mild ataxia 3 plus complete loss of vibratory sense in the lower extremities with moderate to severe ataxia 4 plus ataxic or spastic paraplegia with inability to stand unassisted sometimes associated with sphincter disturbances Evidences of improvement were likewise graded arbitrarily on a plus basis according to which 4 plus signifies complete disappearance of all subjective and objective manifestations Evaluation of degrees of improvement less than complete recovery is dependent upon the

TABLE 2.—*The Maximum Extent of Clinical Improvement in Neurologic Manifestations During Period of Adequate Therapy Related to the Duration of Neurologic Symptoms Before Treatment Was Begun*

Degree of improvement	Number of cases					
	Symptoms present for less than 3 months		Symptoms present for 3 to 12 months		Symptoms present for longer than 12 months	
	Extent of neuropathy					
	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++
None (0)	0	0	1	0	1	0
Slight (+)	0	1	0	1	1	0
Moderate (++)	0	0	4	3	4	6
Marked (+++)	11	2	6	0	2	0
Complete recovery (++++)	3	0	1	0	0	0

TABLE 3.—*The Maximum Extent of Clinical Improvement in Neurologic Manifestations Related to the Type of Therapy Employed During Period in Which Improvement Occurred*

Degree of improvement	Number of cases							
	Desiccated stomach		Oral whole liver and liver extract		Parenteral crude liver extract		Parenteral refined liver extract	
	Extent of neuropathy							
	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++
None (0)	1	0	0	0	1	0	0	0
Slight (+)	0	1	1	0	1	0	0	0
Moderate (++)	4	2	1	2	4	4	0	0
Marked (+++)	5	0	4	0	9	2	2	0
Complete recovery (++++)	1	0	1	0	1	0	0	0

TABLE 4.—*The Neurologic Status After not Less than Ten Years of Observation and Therapy. The Long Term Results Related to the Initial Severity of the Neuropathy and the Adequacy of Treatment as Measured by the Maintenance of Normal Hematologic Values*

Extent of neuropathy	Number of cases																	
	Optimal therapy						Suboptimal therapy without definite relapses						Clinical and hematologic relapses					
	P D	0	+	++	+++	++++	P D	0	+	++	+++	++++	P D	0	+	++	+++	++++
None	0	10	—	—	—	—	0	6	—	—	—	—	4	7	—	—	—	—
Slight and Moderate (+ to ++)	0	1	1	5	8	1	0	1	1	1	4	1	1	1	0	-	3	1
Severe and very severe (+++ to ++++)	0	0	1	7	2	0	0	0	0	1	0	0	0	0	0	0	0	0

P D signifies neurologic manifestations progressed in severity or developed during observation
0 signifies neurologic status remained essentially unchanged
— signifies absence of neuropathy, hence no room for improvement in the disease process

was 68 The lower age range of these patients as compared to that generally re ported for pernicious anemia is of course explained by the fact that they were all followed for at least ten years after the diagnosis was made The average length of the observation period in the case of the males was 13 4 years and for the females was 13 3 years

Objective manifestations of disease of the nervous system were present in 67 1 per cent of the patients in this group Serious disability on a neurogenic basis was present in 15 7 per cent but only 4 patients or 5 7 per cent were unable to stand or walk without assistance It should be pointed out that the low incidence of extremely severe central nervous system disease in this series may be accounted for by the fact that the commonest causes of death in pernicious anemia are the com plications of spinal cord involvement [6] It is of interest however that, in some cases, such fatal complications may be prevented for an apparently indefinite period even though serious disability has been present for a relatively long time before treatment is instituted Although the incidence of neuropathy is approximately the

TABLE 1 —*Extent of Neurologic Manifestations when First Seen*

	Number of cases		
	Males	Females	Total both sexes
None (0)	14	9	23
Slight (+)	10	1	11
Moderate (++)	17	8	25
Severe (+++)	3	4	7
Very severe (++++)	1	3	4
Total	45	25	70

same for the men and women of this series the latter tended to have manifestations of more severe involvement when first seen (table 1) Nevertheless the differences are not sufficiently great and the number of cases is too small to warrant separate consideration of the sexes with respect to the long term course of their disease

The changes in the neurologic status may be correlated with the duration of symptoms referable to the nervous system before institution of therapy (table 2) and with the type of treatment given (table 3) Of special significance for the purposes of this study is the correlation of the long term results with the adequacy of therapy (table 4)

The better outlook for improvement in neural manifestations when these are of short duration was first pointed out by Ungley and Suzman in 1929 [7] has been noted by numerous observers and recently was re emphasized by Rundles [8] The results of treatment of the patients in this series fully support this view but in addition they indicate that even when symptoms have been present for longer than twelve months a considerable degree of improvement will occur in most cases (table 2)

Improvement in the neurologic status was essentially limited in all cases to the first year of treatment and in fact most functional recovery took place during the

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Degree of improvement	Number of cases					
	Symptoms present for less than 3 months		Symptoms present for 3 to 12 months		Symptoms present for longer than 12 months	
	Extent of neuropathy					
	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++
None (0)	0	0	1	0	1	0
Slight (+)	0	1	0	1	1	0
Moderate (++)	0	0	4	3	4	6
Marked (+++)	11	2	6	0	2	0
Complete recovery (++++)	3	0	1	0	0	0

TABLE 3.—*The Maximum Extent of Clinical Improvement in Neurologic Manifestations Related to the Type of Therapy Employed During Period in Which Improvement Occurred*

Degree of improvement	Number of cases							
	Desiccated stomach		Oral whole liver extract		Parenteral crude liver extract		Parenteral refined liver extract	
	Extent of neuropathy							
	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++	+ to ++	+++ to ++++
None (0)	1	0	0	0	1	0	0	0
Slight (+)	0	1	1	0	1	0	0	0
Moderate (++)	4	2	1	2	4	4	0	0
Marked (+++)	5	0	4	0	9	2	2	0
Complete recovery (++++)	1	0	1	0	1	0	0	0

TABLE 4.—*The Neurologic Status After not Less than Ten Years of Observation and Therapy. The Long Term Results Related to the Initial Severity of the Neuropathy and the Adequacy of Treatment as Measured by the Maintenance of Normal Hematologic Values*

Extent of neuropathy	Number of cases																	
	Optimal therapy						Suboptimal therapy with [illegible] relapses						Clinical and hematologic relapses					
	PD	0	+	++	+++	++++	PD	0	+	++	+++	++++	PD	0	+	++	+++	++++
None	0	10	—	—	—	—	0	6	—	—	—	—	4	7	—	—	—	—
Slight and Moderate (+ to ++)	0	1	1	5	8	1	0	1	1	1	4	1	1	1	0	2	3	1
Severe and very severe (+++ to ++++)	0	0	1	7	2	0	0	0	0	1	0	0	0	0	0	0	0	0

PD signifies neurologic manifestations progressed in severity or developed during observation.
0 signifies neurologic status remained essentially unchanged.
— signifies absence of neuropathy, hence no room for improvement in the disease process.

first six months The results are about equal for the different types of therapy employed including desiccated stomach whole cooked liver or oral liver extract and parenteral crude liver extract usually given intravenously (table 3) Because the more refined and concentrated liver extracts were not available when most of the patients were first seen only 2 cases treated initially with such preparations are included in this series However for the past decade refined liver extract given intramuscularly has been employed in the management of most of our new cases of pernicious anemia and the results have been fully equal to those obtained with oral preparations and parenterally administered crude extracts Some of these cases have been included in previous reports [8 9] No patients who received optimal therapy regardless of type suffered exacerbation of their neurologic manifestations

In this series there was no apparent difference over a long period of time with respect to changes in the neurologic status between those patients who received the recommended amount of therapy and whose blood values were consistently within normal limits and those in whom treatment was irregular or was inadequate as judged by variations in erythrocyte count or morphology (table 4) In neither group was development of nervous system disease observed in patients who presented no manifestations of neurologic involvement when therapy was first instituted The incidence and degree of improvement was about the same in the two groups However these observations require comment and the conclusion that irregular or suboptimal therapy provides a safeguard against development of nervous system disease is not justifiable In the first place all of the patients received intensive initial therapy with apparent complete arrest of spinal cord degeneration In the second the fact that these patients returned frequently over a period of many years is evidence that they were cognizant of the importance of adequate follow up examination and treatment even though they at times neglected it Recurrence of paresthesia or of mild symptoms of anemia was a warning to them to resume active therapy In the third place the severity of the neurologic process in the two groups is not comparable Of the 11 patients with evidences of extensive spinal cord involvement 10 are included in the optimal therapy group justifying the inference that irregular or inadequate treatment seriously ffects the chances for long time survival of patients with severe nervous system disease On the other hand it is worthy of note that no patients in the inadequately treated group with the milder degrees of involvement showed more than transient exacerbations of their neurologic manifestations

The patients in this series who suffered definite hematologic relapse as indicated by an erythrocyte count of less than 3 000 000 per cu mm with macrocytosis did not fare as badly as might have been expected Eleven of this group were free of evidence of neural involvement when first seen and only 4 of these developed neurologic manifestations during subsequent relapses In each instance the lesion was classified as moderately severe (++) and was arrested with good functional improvement when intensive therapy was resumed Of 8 patients presenting symptoms and signs of mild or moderate degree only one suffered irreversible progression of spinal cord damage during hematologic relapse Here also it should be

emphasized that the relapses suffered by these patients were generally of short dura tion that the series includes only those patients who were willing and able to re turn and that no patient with pre existing severe central nervous system disease who suffered hematologic relapse has been followed for as long as ten years

Experiences with Folic Acid

Since January 1946 15 patients with pernicious anemia have been treated with synthetic folic acid (pteroylglutamic acid) for sufficiently long periods to permit an evaluation of the early therapeutic results obtained with this material and a comparison of the results with those secured with other forms of treatment Nine members of the group were males 6 or 40 per cent were without evidence of neuro pathy 5 had previously been under treatment for pernicious anemia but only 1 had normal blood values at the time folic acid treatment was begun The 6 patients without nervous system disease have all maintained normal blood values for one year or longer while receiving 5 mg of folic acid by mouth daily and none have developed *neurologic manifestations* *

REPORT OF CASES

One patient (H W) a man of 64 had paresthesias of the extremities diminished knee and ankle jerks and impaired vibratory sense in the lower extremities when folic acid 10 mg orally each day was started during hematologic relapse Within three months coincident with restoration of blood values to *normal paresthesias had disappeared and the patient had no complaints* A woman (T K) aged 69 had evidences of presumptive peripheral nerve and posterior column involvement with slight ataxia and impairment of sense of motion and position when the diagnosis of pernicious anemia was first made during hematologic relapse On folic acid 10 mg daily by mouth there was significant functional and symptomatic improvement of moderate degree observed over a period of eight months A man (C N) aged 6 had moderate involvement of the nervous system when first seen in 1938 He was treated with refined liver extract by intramuscular injection vith marked (+ + +) impro ement in his neurologic status In April 1946 his blood values were slightly abnormal presumably due to too long intervals between treatments and therapy was changed to folic acid 10 mg daily by mouth later reduced to 5 mg There was no reactivation of the neurologic process at the time of institution of folic acid therapy and none has occurred over a p riod of fourteen months A woman (E C) 70 years old was found to have pernicious anemia in 1938 with a moderately severe neural lesion While under treatment with desiccated stomach there was marked (+ + +) improvement in the neurologic manifestations In May 1946 treatment was changed to folic acid 10 mg orally each day After six months there was no exa cerbation of the neurologic process but the erythrocyte level had declined to 3 600 000 per cu mm with a mean corpuscular olume of 105 cubic microns Desiccated stomach 20 Gm daily was substituted for the folic acid and the blood values were rapidly restored to normal

The 4 remaining patients with pernicious anemia in our folic acid treated series may be said to have had unsatisfactory results with respect to their neurologic status In 2 of these the adverse changes were slight and may have been equivocal or the dosage for these individuals may have been too small A physician (W F) was first found to have pernicious anemia in April 1946 and was treated initially with folic acid 15 mg daily by intramuscular injection His erythrocyte count was 2 600 000 per cu mm and the ma imum reticulocyte percentage reached on the eighth day was 15 6 He had troublesome pares thesia especi lly in the toes mild ataxia impaired vibratory s nse distal to the mid tibiae and swaying in the Romberg position After one month on the parenteral 15 mg dosage the patient felt much stronger his appetite had improved and he had gained weight but there was no change in his neurogenic symp-

Since this report was submitted one of the patients developed severe paraplegia while receiving 10 mg of folic acid daily

toms The erythrocyte count was 4 300 000 per cu mm and slight macrocytosis was still present At this time the dosage of folic acid was reduced to 5 mg orally each dav Two months later the neurologic manifestations and the blood values were unchanged He was then given refined liver extract 15 units intramuscularly every three weeks together with the daily oral dose of folic acid 5 mg All neurogenic symptoms except occasional slight tingling in the toes di appeared within two months time and the blood values have been entirely normal for one year A woman (B C) aged 51 was first seen and diag nosed as pernicious anemia in September 1946 Anemia was minimal the erythrocyte count being 3 900 000 but characteristic morphologic changes were present and achlorhydria persi ted after hista mine injection There were manifestations of active moderately severe neurologic disease chiefly paresthesia ataxia and deep sensory disturbances Folic acid orally 10 mg daily was given for two months There was no change in the blood values and the patient stated that numbness and tingling had become more severe although there was no demonstrable alteration in the neurologic signs Folic acid was discontinued and refined liver extract 15 units was given intramuscularly at first twice and later once weekly *The blood values were entirely normal and there was a moderate degree of relief of neuro*genic symptoms one month later

In the other 2 remaining ca es there can be no doubt that spinal cord disease progress d actively while the patients were receiving reasonably large doses of folic acid One of these (J N) a man of 51 years was found to have histamine refractory achlorhydria and atrophic gastritis by gastroscopic ex*amination four years before the diagnosis of pernicious anemia was made At the earlier examination* his complaints were limited to gastrointestinal disturbances he had no glossitis no anemia and no symptoms referable to the n rvous system In April 1946 he was admitted to another hospital where the diagnosi of pernicious anemia was made Symptoms of increasing fatigability paresthesia of the hands and feet and ataxia had been present for about ix months Shortly before his admission he developed pronounced mental changes characterized by depression feelings of guilt and religious preoccupation Details of the hematologic and neurologic examinations at the time of admission are not available but it is known that the anemia was of moderate degree with an erythrocyte count of approximately 3 000 000 p r cu mm The patient was able to walk unassisted and there were no sphincter disturbances He received one or two injections of liver extract and then was treated exclusively with folic acid 20 mg by mouth daily There wa symptomatic improvement with clarification of the mental status and he was discharged after about one month in the ho pital in May 1946 He continued to take folic acid in the above do age at home and the fact that he actually received the medication is attested by his wife who is an entirely reliable person well known to us On June 14 1946 he was first s en in the out patient department of the Simpson Memorial Institute He walked alone but with considerable ataxia was oriented and re spon ive and had no specific complaints other than paresthesia His erythrocyte count wa 3 400 000 p r cu mm hemoglobin 11 9 grams per 100 cc and hematocrit 35 per cent He was advised to continue taking folic acid 20 mg daily On the morning of June 20 he wa unable to lea e his bed and during the next two days he rapidly developed the signs of extremely severe spastic ataxic paraplegia with loss of sphincter control On June 22 he was admitted to the Simpson Memorial Institute Knee and ankle jerks vere not obtained Plantar stimulation gave an extensor response bilaterally Vibratory sense was completely lost over the bones of the lower extremities and the crest of the ilium Sense of motion and position of the toes wa absent The patient was unable to bear any weight on his legs and had no sense of floor resistance Folic a id a di continued and refined liver extract 15 units was given daily by *intramuscular injection This dosage vas continued until August 5 when it was reduced to 15* units three times a w ek until December 12 hen it was changed to 15 units twice weekly Improvement of the neurologic statu as slow but definite After six eeks he was able to use a walking device and sphincter control had returned In four months he could get about ith crutches and nine months after the institution of li er extract therapy he discarded the crutches for canes At that time March 1947 the knee and ankle jerks had returned the Babinski sign was no longer obta ned and vibratory sense as present although diminished as far distal as the mid tibiae Hematologic values ere restored to normal within a few weeks after beginning liver therapy

The last case to be reported is that of a man (S K) of 55 years ho was admitted to ou service on July 12 1946 His earliest symptom was impaired sense of taste (? olfactory distu bance) and anorexia which developed eleven months before his admission One month later he first noted numbness and t n gling of the extremities and difficulty in valking His symptoms were very slowly progressive and he

continued with his occupation as a merchant until his hospital admission The initial red blood cell count was 3 600 000 per cu mm hemoglobin 12 2 grams per 100 cc hematocrit 36 per cent Histamine refractory achlorhydria was present The gait was ataxic Romberg and Babinski signs were present Knee and ankle jerks were hyperactive bilaterally vibratory sense was impaired to absent over the lower extremities and sense of motion and position of the toes was disturbed but not completely lost A diagnosis of pernicious anemia with moderately advanced postero-lateral column degeneration was made and the patient was treated with folic acid 10 mg intramuscularly daily After ten days the oral route was substituted for the intramuscular mode of administration The patient was discharged on July 28 and walked out of the hospital unassisted He returned eleven days later on August 9 unable to stand or walk alone and with complete loss of vibration and position sense in the lower extremities The erythrocyte count at this time was 3 900 000 per cu mm hemoglobin 14 6 grams per 100 cc hematocrit 39 per cent In place of folic acid he was given refined liver extract 15 units intramuscularly daily for one week then 15 units three times a week He returned four weeks later on September 9 showing some improvement but unable to walk without assistance At his next visit after another interval of four weeks he walked alone with the aid of a cane On June 17 1947 after ten months of liver extract therapy he felt quite well and walked with only a slightly hesitant gait Vibratory sense was apparently normal over the right lower extremity but was diminished although nowhere absent over the left There was return of sense of motion and position of the toes

Discussion

Experience in the management of cases of pernicious anemia during the early period of their treatment and over a number of years indicates that administration of sufficient amounts of desiccated stomach whole liver oral liver extract and crude or refined liver extracts by parenteral routes if accompanied by a hematologic response, will invariably lead to the arrest of the neurologic degenerative process and will usually be followed by a significant degree of symptomatic and functional improvement Furthermore if adequate treatment as judged by consistently normal erythrocyte values is taken continuously exacerbation of nervous system disease will not occur Even if treatment is irregular and suboptimal provided there are no long periods of relapse patients with less severe degrees of neurologic involvement will rarely suffer irreversible progression of their neural lesion These statements are apparently not valid in the case of folic acid therapy The occurrence and progression of combined system degeneration in patients with pernicious anemia under treatment with folic acid was first reported by Vilter Vilter and Spies[10] and by Meyer [11] The phenomenon has also been noted by Heinle and Welsh[12] and by Hall and Watkins [13] In some of the cases reported the activity of the process was not arrested when the dosage of folic acid was increased many times

The failure of folic acid to control spinal cord disease in some cases may be taken as evidence that this vitamin corrects only a specific deficiency responsible for the hematopoietic disturbances occurring in pernicious anemia This hypothesis however does not satisfactorily explain two types of observations which have been made by ourselves as well as by others namely first the fact that not all patients with pernicious anemia in the absence of important complications respond to folic acid by restoration of fully normal hematologic values even when large doses of the vitamin are given by both oral and parenteral routes and second some patients experience definite relief of neurogenic symptoms while receiving folic acid This improvement may be due primarily or entirely to peripheral nerve

recovery but even so it may be said that such patients, over a period of many months show no evidence of progress of nervous system involvement

The fairly uniform and predictable results of liver and stomach therapy in pernicious anemia and the variability of the responses of persons with this disease to administration of synthetic folic acid suggest that there is in pernicious anemia a widespread metabolic defect in which a number of interrelated factors or processes are involved one of these being the ability to convert the naturally occurring conjugated form of folic acid to the free vitamin [14] [15] However defective utilization of folic acid is not the only cause either of the hematopoietic or the neural disturbances It appears, with some supportive evidence [14] that among the therapeutic properties of stomach and liver is included a corrective effect on the disordered metabolism of folic acid which is responsible in part for the manifestations of pernicious anemia

Summary and Conclusions

Seventy patients with pernicious anemia have been observed for periods of not less than ten years The clinical course in these cases has been analyzed with particular reference to changes in the neurologic status

Most of the patients whether treated with oral preparations of stomach or liver or parenteral crude or refined liver extracts showed significant improvement of their neurologic manifestations The period of improvement was limited essentially to the first year of therapy

Thirty six members of the series received treatment regularly and were maintained consistently in complete hematologic remission Fifteen of the patients did not adhere to an optimal therapeutic regimen and their blood values were frequently abnormal although definite relapses did not occur In the former group there were no instances of development or progression of neural lesions In the latter such adverse changes as did occur were transient and reversible on resumption of adequate therapy Nineteen patients in the series suffered clinical and hematologic relapses after their initial response to intensive therapy The end results in this group were not so favorable but nevertheless serious progression of spinal cord involvement was rarely observed The apparent infrequent occurrence of pronounced changes is attributed to the short duration of the relapses and to the relatively mild degree of nervous system involvement present when the diagnosis of pernicious anemia was made It may be assumed that patients with more extensive neural disease who suffered relapses progressed to a fatal termination

The observations reported in no way justify the conclusion that irregular or suboptimal therapy is without serious risk They are presented in order to indicate what the long term clinical results may be in the case of patients with pernicious anemia who frequently fail to adhere to an ideal therapeutic regimen

The early results of treatment with synthetic folic acid as observed in a series of 15 patients indicate that both the hematologic and neurologic response to this form of therapy is much less predictable than is the case with stomach or liver preparations It is suggested that disturbance of folic acid metabolism is not the sole cause of either the hematologic or the neurologic manifestations of pernicious

anemia but that inability to utilize folic acid effectively may play a part in the development of both myeloid and neural abnormalities

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THE DEVELOPMENT AND PROGRESSION OF SUBACUTE COMBINED DEGENERATION OF THE SPINAL CORD IN PATIENTS WITH PERNICIOUS ANEMIA TREATED WITH SYNTHETIC PTEROYLGLUTAMIC (FOLIC) ACID

By J F Ross M D* H Belding M D, and B L Paegel M D

THE ISOLATION identification and synthesis of folic acid (pteroylglutamic acid) have provided an extremely potent hematopoietic agent [1] Unquestionably folic acid induces hematologic remissions in patients with pernicious anemia Evidence is accumulating that indicates its ineffectiveness in preventing the development or progression of subacute combined degeneration of the spinal cord Furthermore it is not certain that normal blood levels can be maintained for prolonged periods of time in patients treated with folic acid alone

During the last seventeen months we have substituted synthetic folic acid† for liver extract in the treatment of 22 patients with pernicious anemia These cases have been observed with extreme care for evidences of development or progression of neurologic complications and for changes in hematologic or clinical status During this brief period of time significant changes have developed in many of these patients that make advisable a report of our observations at the present time

Method of Study

CLINICAL MATERIAL

The diagnosis in each case was established by the demonstration of a macrocytic hyperchromic anemia with an associated leucopenia and thrombocytopenia a histamine refractory gastric achlorhydria and a response to liver extract or to folic acid with reticulocytosis and restoration of normal blood values In many cases a megaloblastic bone marrow typical of pernicious anemia was demonstrated before treatment was started Four patients were in hematologic relapse and were hospitalized during the initial period of study Two of these patients previously had been seen in hematologic relapse and remission had been induced with liver extract It has been possible to compare their responses to folic acid with those previously obtained with liver extract One patient in relapse had severe active subacute combined degeneration of the spinal cord

The patients in remission had been known to have pernicious anemia for periods ranging from one to nineteen years and had been treated with liver extract in the Outpatient Department of the Massachusetts Memorial Hospitals before the beginning of this study Six of these patients had evidence of subacute combined degeneration as manifested by paresthesias diminution of vibration sense or reflex changes at the beginning of the study but in all these cases the disease had been arrested and the signs had been unchanged for several years Liver extract therapy was stopped at the beginning of this study There was no restriction of diet which was considered to be adequate in all patients Eleven patients were taking supplementary vitamins or yeast and these were allowed to continue this medication during the period of study Folic acid was administered orally in 16 cases in doses ranging from 1 25 to 15 0 mg daily Six patients received 30 to 100 mg by intramuscular injection once every four weeks

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* Formerly Welch Fellow in Internal Medicine of the National Research Council A portion of this investigation was carried out while under tenure of the Welch Fellowship

† The synthetic folic acid (pteroylglutamic acid) used in this study was kindly furnished by Dr Stanton M Hardy of the Lederle Laboratories Inc Pearl River N Y

All the patients were seen at least monthly in the Outpatient Department of the Massachusetts Memorial Hospitals and when it became evident that neurologic symptoms were developing they were seen at weekly or semiweekly intervals Careful neurologic and hematologic studies were performed at each visit

TECHNICAL METHODS

Blood studies were performed on venous blood placed in mixed ammonium and potassium oxalate [1] Hemoglobin determinations were done on photoelectric colorimeters by the oxyhemoglobin method [2] Hematocrits were determined with Wintrobe tubes with centrifuging for one hour at a relative centrifugal force of 1800 Erythrocyte and leucocyte counts were made in duplicate and averaged Erythrocyte indices were calculated by the method of Wintrobe Direct platelet counts were made with Rees-Ecker diluting fluid Reticulocyte counts were made on blood films prepared from a mixture of venous blood with 0 3 per cent cresyl blue and 0 6 per cent solution of sodium chloride Films of bone marrow obtained by sternal aspiration were stained with Wright s and Giemsa s stain

OBSERVATIONS

PERNICIOUS ANEMIA IN RELAPSE

Four patients were in severe hematologic relapse when folic acid therapy was started Of these 2 had never before had antianemia therapy 1 was in relapse subsequent to three years without liver extract therapy and 1 was in relapse following a low maintenance dose of folic acid The courses of these 4 patients are typical of the responses that may be produced with folic acid and brief case histories are presented

CASE 1

Pernicious anemia in severe hematologic relapse Remission induced with orally administered folic acid Gradual development of anemia and subacute combined degeneration of the spinal cord after one year of maintenance on folic acid

J B a 65 year old white man was admitted to the hospital in May 1946 with a history of easy fatigability for two years and marked weakness and anorexia for two weeks Physical examination revealed pallor of the skin and mucous membranes a red tongue with atrophy of the lateral papillae hypoactive deep tendon reflexes in the lower extremities and slightly diminished vibration sense in the feet Blood studies showed a severe macrocytic anemia leucopenia and thrombocytopenia (table 1 and fig 1) and the sternal bone marrow was characteristic of pernicious anemia in relapse There was histamine refractory gastric achlorhydria Folic acid therapy 15 mg daily by mouth was started at this time Weakness and anorexia were noticeably lessened on the second day of therapy and the patient was completely asymptomatic at the end of the second week In one month s time the physical findings were normal and there was full recovery of vibration sensation A maximum reticulocyte response of 29 per cent was reached on the eighth day of therapy Just prior to the rise in reticulocytes a substantial increase in the white cell and platelet counts was noted A steady increase in the red cell count and hemoglobin began after the fourth day Normal blood levels and red cell indices were present by the third month and were maintained until the eighth month when the red cell count hematocrit and hemoglobin began to fall By the twelfth month the patient showed a definite anemia

With the exception of occasional slight glossitis the patient remained asymptomatic during the first eleven months of folic acid therapy Beginning with the seventh month there was gradually progressing diminution in vibration sense although position sense and motion sense were normal and Romberg s sign was negative In the twelfth month the patient began to experience stiffness of the feet and numbness of the fingers His gait became unsteady particularly in the evening and he staggered when he attempted to walk in the dark Knee and ankle jerks were markedly hypoactive at this time vibration sense was

impaired below the knees and motion sense was diminished in the toes The patient swayed slightly in the Romberg position and staggered when he tried to walk with his eyes closed

Comment This previously untreated case of pernicious anemia showed early and rapid clinical and hematologic improvement in response to 15 mg of folic acid given daily by mouth The reticulocytosis of 29 per cent in response to folic acid therapy was slightly less than the 37 per cent expected optimum response to liver extract but the rate of regeneration of erythrocytes, leucocytes and platelets was fully as rapid as would have been expected with liver extract therapy The blood remained normal for eight months but subsequently fell to anemic levels Definite subjective and objective signs of subacute combined degeneration appeared during the twelfth month of folic acid therapy

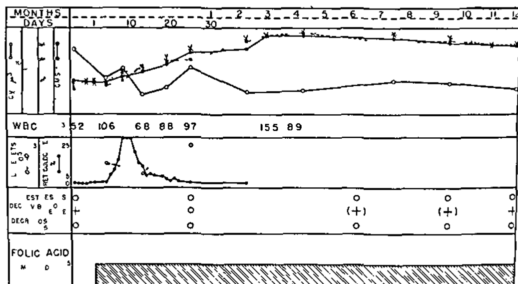


FIG 1 (Case 1) Previously untreated pernicious anemia in relapse Remission induced with orally administered folic acid Gradually developing anemia and subacute combined degeneration after 7 month of folic acid therapy

CASE 2

Pernicious anemia with mild anemia but severe subacute combined degeneration No improvement in neurologic status after twenty five days of orally administered folic acid

A W a 60 year old white woman was admitted to the hospital with a history of increasing paresthesias in all extremities weakness anorexia and weight loss of one year s duration The significant physical findings were slight atrophy of the lateral lingual papillae hyperactive deep tendon reflexes a positive Romberg s sign a slightly ataxic spastic wide based gait marked diminution of vibration sense in the knees ankles toes and fingers and some impairment of position sense in the fingers The red cell count was 3 000 000 the hemoglobin 12 8 Gm per 100 cc the mean corpuscular volume 122 cubic micra and the white cell count 5700 There was histamine refractory gastric achlorhydria Folic acid was administered orally in a daily dose of 15 mg throughout the twenty days of hospitalization The reticulocytes rose from 2 1 to 5 8 per cent on the seventh day At discharge the red cell count was 3 410 000 the hemoglobin 13 9 Gm per 100 cc and the white cell count 8125 After one week during which the

folic acid dosage was increased to 45 mg daily the red cell count was 3 540 000 and the hemoglobin 14 Gm per 100 cc

After twenty-eight days of folic acid therapy paresthesias and weakness persisted unchanged and there was no improvement in the signs of subacute combined degeneration of the spinal cord Daily intramuscular injections of 15 units of purified liver extract were begun and continued during the next five weeks Subjective improvement and a decrease in paresthesias occurred in response to this therapy but further neurologic examinations could not be performed A blood study performed after two months of folic acid therapy and thirty-eight days of liver extract therapy showed a normal hemoglobin and hematocrit but persistent marked macrocytosis (table 1)

Comment This previously untreated patient had a moderate although markedly macrocytic anemia but severe subacute combined degeneration of the spinal cord She had a submaximal reticulocyte response to folic acid (only 5 8 per cent as compared with an anticipated 12 0 per cent) and the blood levels rose very slowly even after liver extract therapy was given in addition to an increased dosage of folic acid There was no subjective or objective evidence of improvement of the subacute combined degeneration during twenty-eight days of folic acid treatment and it was not possible to follow the patient adequately for a longer period of time

CASE 3

Pernicious anemia in relapse remission induced with orally administered folic acid Subacute combined degeneration and mild anemia developing after sixteen months of maintenance therapy with folic acid Progression of subacute combined degeneration after institution of liver extract therapy while folic acid was continued

H C a 59 year old white woman was first admitted to the hospital in December 1940 with weakness glossitis anorexia and weight loss of two years duration Physical examination revealed marked pallor of the skin and mucous membranes atrophy of the lingual papillae moderate glossitis a palpable liver and diminished vibration sense below the knees There was a severe macrocytic anemia and a megaloblastic bone marrow (table 1 and fig 2) Histamine refractory gastric achlorhydria was present In response to 15 units of liver extract administered intramuscularly daily there was a rise in reticulocytes to 14 6 per cent After one month of therapy the red cell count was 4 330 000 and red cell indices were essentially normal Therapy was gradually reduced to 15 units of liver extract a month Blood values continued to rise and throughout the ensuing seventeen months were optimal

Except for the occasional ingestion of cooked liver the patient lapsed in therapy from September 1942 to January 1946 when she was readmitted to the hospital with recurrence of the original symptoms and paresthesias of the hands and feet The significant physical findings were pallor of the skin and mucous membranes atrophy of the lateral lingual papillae a slightly unsteady gait hyperactive knee jerks absent ankle jerks and a moderate decrease in vibration sense below the knees The blood and bone marrow were characteristic of pernicious anemia in relapse (table 1 and fig 2) The patient was given 15 mg of folic acid by mouth daily and a maximum reticulocyte response of 11 8 per cent was obtained on the sixth day of therapy She rapidly became asymptomatic and after one month of treatment the red cell count and hemoglobin had risen to moderate levels with a decrease in macrocytosis There was no change in neurologic status at that time Folic acid therapy (15 mg by mouth daily) was continued after discharge in February 1946 One month later the patient was readmitted for treatment of a myocardial infarction and pyelitis Vibration sense in the ankles and toes was diminished The blood values were unchanged The dosage of folic acid was increased to 100 mg daily by mouth for five days and then intravenously for the next ten days This increased dose produced no improvement in the blood picture and the original dosage of 15 mg daily by mouth was resumed Nineteen days later this was supplemented by daily intramuscular injections of 15 units of liver extract for twelve days This resulted in symptomatic improvement relief of the glossitis and improvement in vibration sense in the ankles and toes but no

further hematologic response During the subsequent two months on folic acid therapy alone the red cell count and hemoglobin rose to fairly satisfactory levels and the red cell indices became normal Vibration sense was fully recovered During the ensuing ten months there was a gradual decrease of the red cell and hemoglobin levels and a return of macrocytosis

In the sixteenth month of folic acid treatment and twelve months after the last injection of liver extract the patient complained of steadily increasing numbness of the hands forearms and feet Her family stated that she became extremely forgetful and irritable There was a rapid and progressive loss of vibration sense in the lower extremities and impairment of position and motion sense in the toes and fingers *Liver extract therapy was started 75 units being given first semiweekly and then weekly* and folic acid medication was continued In spite of the injection of 260 units of liver extract during the next six weeks the symptoms and signs of subacute combined degeneration of the spinal cord progressed At the end of this period folic acid therapy was discontinued

FIG 2 (Case 3) Pernicious anemia with comparable remissions induced on different occasions with liver extract and with folic acid Suboptimal response to oral folic acid not improved by large doses of folic acid administered intravenously or by liver extract Progression of subacute combined degeneration 12 months after last injection of liver extract

Comment This case provides an interesting comparison of the relative effectiveness of folic acid and liver extract in inducing remission in pernicious anemia Symptomatic improvement occurred earlier and was more marked with liver extract than with folic acid therapy The reticulocyte response to both liver extract and folic acid was submaximal The rate of increase in the red cell count in response to folic acid was slower than had occurred previously with liver extract but the hematologic response probably was hampered by the complicating myocardial infarction and pyelitis In the face of these complications neither an increase in the dosage of folic acid nor a course of liver extract therapy was effective in improving the blood picture Following recovery from these illnesses the blood levels temporarily rose to normal The optimal levels previously maintained with liver extract therapy never were observed during folic acid treatment and a gradual diminution of blood levels occurred during the last ten months of therapy Subacute combined de

generation became apparent in the sixteenth month of folic acid therapy, twelve months after the last injection of liver extract and neurologic disease progressed during the next six weeks in spite of the injection of 260 units of liver extract

CASE 22

Hematologic relapse on 30 mg of folic acid administered once monthly by injection Remission on 15 mg of folic acid daily by mouth Subsequent explosive development of severe subacute combined degeneration of the spinal cord

J S a 69 year old white man had been treated for pernicious anemia with liver extract by a private physician in 1938 and was first admitted to the hospital in 1939 with a history of weakness and anorexia following a six months lapse in therapy Blood studies showed a severe macrocytic anemia (table 1 and

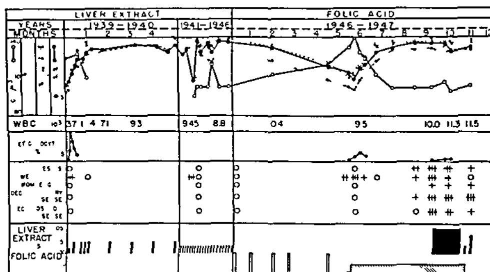


Fig 3 (Case 22) Relapse of pernicious anemia while receiving 30 units of folic acid by intramuscular injection once each month Remission induced with 15 mg of folic acid daily by mouth comparable with remission induced with liver extract on previous occasions Rapid development and progression of sub acute combined degeneration on folic acid maintenance therapy while blood was normal

fig 3) The patient responded to liver extract therapy with a reticulocyte response of 20 9 per cent on the sixth day of therapy and normal blood levels were attained by the third month In 1942 the patient again lapsed in therapy and underwent a second hematologic relapse He again showed an adequate response to liver extract Since 1943 adequate blood levels were maintained with intramuscular injection of 30 units of liver extract monthly The patient ingested daily 10–15 Gm of yeast during the entire period of the subsequent study a fact that should exclude vitamin deficiency as a contributing factor Folic acid was substituted for liver extract in June 1946 and 30 mg of folic acid once each month was given by intra muscular injection At this time the patient was completely asymptomatic physical examination was essentially negative and neurologic examination was entirely negative The blood levels were sub optimal with a moderate degree of macrocytosis (table 1 and fig 3) There was a persistent drop in the red cell and hemoglobin levels and by the fifth month the patient showed a marked macrocytic anemia Weakness and dyspnea appeared during the third month but the only abnormal physical finding was marked pallor of the skin and mucous membranes Neurologic examination was completely negative At this time therapy was changed to a daily oral dose of 5 mg of folic acid Symptoms of anemia increased

further hematologic response During the subsequent two months on folic acid therapy alone the red cell count and hemoglobin rose to fairly satisfactory levels and the red cell indices became normal Vibration sense was fully recovered During the ensuing ten months there was a gradual decrease of the red cell and hemoglobin levels and a return of macrocytosis

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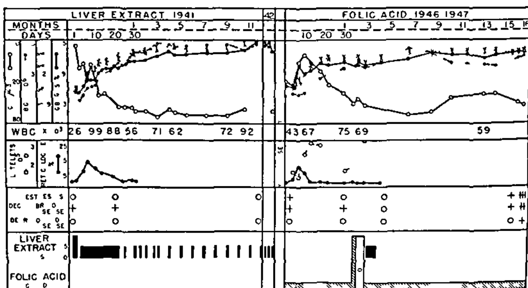


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Comment This patient gradually developed a hematologic relapse over a six month period during which treatment consisted of 30 mg of folic acid by injection once a month The rate of development and the severity of the hematologic relapse were comparable to two previous relapses that occurred following omission of liver extract This indicates that folic acid in the amount given had no effect in maintaining remission The hematologic response to 15 mg of folic acid taken orally each day was excellent and was comparable with the response obtained on two previous occasions with liver extract

The most striking features of this case were the explosive development of extremely severe subacute combined degeneration after nine months of folic acid therapy and its appearance at a time when the peripheral blood picture and bone marrow were normal Improvement of symptoms and signs of lateral column disease occurred gradually following the addition of liver extract therapy but vibration sense and numbness of the hands and feet improved only after discontinuation of folic acid

The clinical and hematologic responses of 3 cases to folic acid therapy were excellent and quite comparable to those that would have been expected with optimum liver extract therapy Indeed in Case 22 the rate of blood regeneration under folic acid therapy almost exactly paralleled that previously obtained with liver extract (fig 3) The reticulocyte responses were slightly less than would have been expected with liver extract but were quite adequate in all 3 cases Increases in white cell and platelet counts were marked and rapid In Case 2 the degree of anemia was not severe and consequently the rate of blood regeneration was not rapid The suboptimal blood levels eventually obtained in Case 3 were not improved by additional liver extract therapy and probably are a reflection of the complicating cardiac disease and pyelonephritis

Cases 1 3 and 22 showed improvement in the sense of well being on the third or fourth day following the beginning of folic acid therapy This improvement was neither so striking nor so marked as that usually seen following the exhibition of liver extract Glossitis was present in Cases 1 3 and 22 and improved very slowly under folic acid treatment In Case 1 it occasionally recurred during the period of maintenance therapy

The extremely marked signs of subacute combined degeneration in Case 2 showed no improvement during the twenty-eight days of folic acid therapy but they did not progress The numbness and slight diminution of vibration sense initially present in Cases 1 and 3 gradually improved over a period of several months with folic acid therapy but recurred again after a year s treatment Case 22 showed no neurologic abnormality at the time of hematologic relapse and it was not until three months later at a time when the blood picture was normal that the patient developed extremely severe and rapidly progressive subacute combined degeneration of the spinal cord

Maintenance Therapy of Pernicious Anemia in Remission

Twenty one patients with pernicious anemia in remission have been maintained on folic acid therapy for periods ranging from eight to seventeen months In 19

and there was a further decrease in blood levels during the next week The dosage of folic acid was in creased to 15 mg a day by mouth Rapid symptomatic and hematologic improvement followed and in two months the red cell count returned to its original pre folic acid level A reticulocyte response of 6 3 per cent was noted on the seventh day but since these counts were performed only at weekly intervals the maximum reticulocytosis probably was not detected Neurologic examination at this time was negative

Eight and one half months after folic acid had been substituted for liver extract three months after the start of oral folic acid therapy and one month after restoration of normal blood levels the patient com plained of *heaviness in the soles of his feet and stated that he felt muscle bound in the calves and thighs* The only neurologic abnormality at this time was slightly diminished vibration sensation in the toes One week later his hands and feet felt like blocks of wood and there were periodic episodes of a burning sensation from the toes to the hips At this time the patient began to need assistance in maintaining his balance and soon he was unable to walk without considerable aid Within another two weeks he was unable to stand or walk and could not feed himself because of incoordination of the hands

On admission to the hospital the general physical examination complete blood studies and sternal bone marrow examination yielded normal findings The patient s mental status and speech were normal Examination of the cranial nerves showed impairment in the first (inability to recognize the smell of coffee vanilla or wintergreen) and the second (moderate bilateral diminution in visual acuity and mod erate constriction of visual fields) although the optic fundi were not remarkable The remaining cranial nerves were intact There was no gross inequality in muscular volume or power but there was a definite increase in muscle tone spastic in type of the arms and legs Coordination was extremely poor and the patient was unable to hold objects or to feed himself There was astereognosis even for large objects and marked past pointing in both arms The heel to-shin test was poorly done Romberg s sign was positive and the gait even with assistance was spastic slapping and staggering Deep tendon reflexes in the lower extremities were markedly hyperactive more so on the left than on the right The Babinski response was *present on the left and there was no response to plantar stimulation on the right* Abdominal and crem asteric reflexes were absent Vibration sense was entirely absent below the tenth thoracic segment and in both hands wrists and elbows Position and motion sense were impaired generally most markedly in the fingers toes and ankles There was loss of light touch sensation over the ulnar side of both hands and on the medial aspect of the right foot There was irregular patchy diminution of pain and temperature sensa tion in both arms and both legs and decrease in sweating of both lower extremities and complete absence of sweating from the level of the knees downward There was no interference with bladder or bowel function however and no change in sexual function (the patient had been impotent for 10 years) A lumbar puncture yielded normal spinal fluid

Injection of 105 units of concentrated liver extract (15 units per cubic centimeter) was given intra muscularly daily for the next five weeks and the administration of 15 mg of folic acid daily by mouth was continued During this period there was gradual subjective improvement beginning on the eleventh day The patient gradually regained the coordinated use of his hands and feet and after three weeks of liver extract therapy he was able to feed himself and to walk unassisted and without staggering although the gait was still spastic and slapping He was discharged from the hospital at the end of five weeks at which time he felt well except for some residual numbness of the hands and feet and stiffness of the knees Co ordination of movement was normal but the gait showed slight slapping During the period of hos pitalization the pattern of neurologic improvement was striking First there was a decrease in the signs of lateral column disease muscle tone returned to normal deep tendon reflexes became less hyperactive the plantar responses became flexor and there was a gain in strength With this improvement the patient was able to walk and to feed himself even though the posterior column signs still persisted Position sense was slightly improved in the fifth week of liver extract therapy but there was no return of vibration sense

Following discharge the patient was given 75 units of liver extract intramuscularly once a week and the dosage of 15 mg of folic acid daily was continued There was only slight improvement in position sense during the next three weeks no improvement in vibration sense and persistence of paresthesias Folic acid was discontinued after two months of liver extract therapy Two weeks later position sense was practically normal vibration sense perception began to return and the hands and feet felt much less numb and nearly normal

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Maintenance Therapy of Pernicious Anemia in Remission

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TABLE 1 —Clinical Course of Patients Treated with Synthetic Folic Acid*

Case	Name	Age	Sex	Initial Diagnosis	Duration of Illness before Folic Acid	Previous Treatment with Liver Extract	Folic Acid Therapy: Duration of Therapy or Time in Relation to Start	Dosage: Mg	Dosage: Frequency	Dosage: Route	Blood Findings: Red cell count	Hemoglobin	Hematocrit	MCV	White-cell count	Clinical Observations: Weakness	Glossitis	Paresthesias	Vibration sense	Position sense	Incoordination	Knee jerks	Ankle jerks	Babinski sign	Romberg sign	Abnormal gait
					yr	units per month					× 10⁶	gm/100 cc	%	cu μ	× 10³											
1	J B	65	M	P A	2	None	Before				1.37	5.3	16.1	117	6.45	++++	++	++	--	N	N	--	--	N	N	N
							4 mo	15	daily	oral	4.90	14.0	45.0	9	8.95	N	+	N	N	N	N	N	N	N	N	N
							7	15	daily	oral	4.46	13.9	44.0	99		N	N	N	-	N	N	N	N	N	N	N
							12 mo	15	daily	oral	4.14	12.1	39.0	94	8.3	N	+	+	--	-	N	-	-	N	N	+
2	A W	60	F	P A S C D	1	None	Before				3.00	12.8	36.5	122	5.7	+++	++	++++	----	--	++++	+++	+++	N	+++	++
							20 d	15	daily	oral	3.41	13.9	41.0	120	8.3	++	++	++++	----	--	++++	+++	+++	N	+++	++
							8 d	45	daily	oral	3.54	14.0	42.5	120		+	+	++++	----	--	+++	+++	+++	N	++	++
							36 da	45 +15 units liver extract	daily daily	oral I M	4.06	15.4	46.1	114	7.3	+	N	++	?	?	?	?	?	?	?	N
3	H C	39	F	P A S C D	5	15 Lapse in treatment for 3 years	Before				1.55	6.9	18.9	122	4.28	+++	++	++	--	N	N	++	-	N	N	+
							53 d	15	daily	oral	3.59	11.3	34.8	97	10.8	N	+	N	--	N	N	+	-	N	N	N
							5 da	100	daily	oral	3.60	11.3	36.1	100	5.0	N	+	N	--	N	N	+	-	N	N	N
							10 da	100	daily	I V	3.42	10.4	33.1	97	6.95	N	+	N	--	N	N	+	-	N	N	N
							19 da	15	daily	oral		11.2	36.3			N	+	N	--	N	N	+	-	N	N	N
							12 da	15 +15 units liver extract	daily daily	oral I M		11.4	36.4		7.2	N	N	N	N	N	N	N	N	N	N	N
							6 mo after start	15	daily	oral	4.77	12.0	40.5	85		N	N	N	N	N	N	N	N	N	N	N
							10 mo	15	daily	oral	3.79	12.7	39.0	103		N	N	N	N	N	N	N	N	N	N	N
							16 mo	15	daily	oral	4.07	13.4	39.0	96		++	N	++	N	N	N	N	N	N	N	N
							16½ mo	15 +15 units liver extract q 2 wks	daily	oral I M	4.19	13.4	39.5	94		++	+	+++	--	--	+++	+	N	N	N	+
							17 mo	15 +75 units liver extract weekly	daily	oral I M	3.86	12.7	39.0	101	8.95	++	N	+++	--	-	+++	++	++	N	+++	++
							17½ mo	folic acid omitted 75 units liver extract weekly		I M	4.24	13.4	42.0	96		++	N	+++	--	-	+++	++	++	N	++++	+++

4	R S	67	M	P A S C D gas tr tomy	16	30	B f e				3 0	13 3	42 5	115	5 3	N	N	+	--	N	N	N	-	N	N	N
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							8 mo	15	d ly	1	4 91	14 3	44 0	90	8 1	N	N	N	--	N	N	N	-	N	N	N
							11 m	15	da ly	o al	4 32	14 0	42 0	97		+	N	++	--	N	++	++	-	+	+	+
							12 mo	15	da ly	1	4 31	14 2	42 0	97	7 8	+	N	++	---	-	++	++	-	+	++	++
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							12½ mo	15	da ly	1	4 40	14 1	42 0	95	8 8	++	N	+++	----	--	+++	+++	-	++	+++	+++
								+105 e t	u t l t da ly	I M																
5	K H	75	F	P A	3	30	Bef				3 69	12 9	38 5	104	8 2	N	N	N	N	N	N	N	N	N	N	N
							3 mo	15	da ly	1	4 79	13 3	43 0	90		N	N	N	N	N	N	N	N	N	N	N
							8 m	15	d ly	1	4 8	13 1	41 5	87		N	N	N	N	N	N	N	N	N	N	N
							12 m	15	d ly	oral	4 02	11 6	38 5	96	7 2	N	N	N	-	N	N	N	N	N	N	N
6	A M	60	M	I A	7	30	B f e				4 17	12 9	40 6	97	10 9	N	N	N	N	N	N	N	N	N	N	N
							2 m	15	da ly	1	4 12	13 7	41 2	100	9 8	N	N	N	N	N	N	N	N	N	N	N
							8 m	15	d ly	al	4 32	12 1	37 0	86	9 2	N	N	N	N	N	N	N	N	N	N	N
7	J Mc	66	M	P A	19	30	Bef				2 66	10 2	31 5	118	6 2	N	N	N	N	N	N	N	N	N	N	N
							3 mo	10	d ly	1	3 96	14 0	40 5	102		N	N	N	N	N	N	N	N	N	N	N
							6 mo	10	d ly	1	3 25	12 6	37 0	114		N	N	N	N	N	N	N	N	N	N	N
							12 m	10	da ly	oral	2 89	10 6	31 0	107	5 4	N	N	N	N	N	N	N	N	N	N	N
8	A B	64	M	P A	4	30	B fo e				4 43	13 4	41 0	93	6 0	N	N	+	N	N	N	N	N	N	N	N
							3 m	10	d ly	1	4 12	13 1	39 0	95		N	N	N	N	N	N	+	N	N	N	N
							7 m	10	da ly	oral	4 03	12 8	39 5	98		N	N	+	-	N	N	++	++	+	N	N
							12 mo	10	da ly	oral	4 11	13 9	40 0	98	7 2	N	N	+	--	N	N	+++	++	+	+	N
9	J M	83	M	P A	7	30	B f re				3 93	13 4	40 5	103	7 6	N	N	N	N	N	N	N	N	N	N	N
							3 m	5	d ly	1	4 55	14 6	43 5	96		N	N	N	N	N	N	N	N	N	N	N
							8 mo	5	da ly	1	5 13	15 1	44 0	86		N	N	N	N	N	N	N	N	N	N	N
							12 mo	5	daily	o l	3 88	13 2	40 5	104	8 3	N	N	N	N	N	N	N	N	N	N	N
10	F S	73	M	P A	7	15	B f e				3 97	14 4	45 0	114	6 7	N	N	N	N	N	N	N	N	N	N	N
							2 mo	5	d ly	or 1	4 54	14 6	44 0	97	7 6	N	N	N	N	N	N	N	N	N	N	N
							6 mo	5	daily	or 1	4 69	15 2	45 5	97		N	N	N	-	N	N	-	-	N	N	N
							12 m	5	da ly	oral	4 85	14 4	44 0	91	7 8	N	N	++	--	N	N	--	--	+	+	+

N = o m l   + to ++++ = i c e sed   - t ---- = d crea ed to ab nt   P A = pern i s an mia   S C D = sub te comb d l g ne ati n
Add t al r ma ks page 80

TABLE 1 —*Clinical Course of Patients Treated with Synthetic Folic Acid**

Case	Name	Age	Sex	Initial Diagnosis	Duration of Illness before Folic Acid	Previous Treatment with Liver Extract	Duration of Therapy or Time in Relation to Start	Folic Acid Therapy — Dosage: Mg	Frequency	Route	Blood Findings: Red cell count	Hemoglobin	Hematocrit	MCV	White-cell count	Clinical Observations: Weakness	Glossitis	Paresthesias	Vibration sense	Position sense	Incoordination	Knee jerks	Ankle jerks	Babinski's sign	Romberg's sign	Abnormal gait
					yr	[illegible]					× 10^6	gm/100 cc	%	cubic microns	× 10^3											
1	J B	65	M	P A	2	N	Before				1 37	5 3	16 1	117	6 45	++++	++	++	--	N	N	--	--	N	N	N
							4 mo	15	daily	oral	4 90	14 0	45 0	92	8 95	N	+	N	N	N	N	N	N	N	N	N
							7 mo	15	daily	oral	4 46	13 9	44 0	99		N	N	N	-	N	N	N	N	N	N	N
							12 mo	15	daily	oral	4 14	12 1	39 0	94	8 3	N	+	+	--	-	N	-	-	N	N	+
2	A W	60	F	P A S C D	1	N	Before				3 00	12 8	36 5	122	5 7	+++	++	++++	----	--	++++	+++	+++	N	+++	++
							20 d	15	daily	oral	3 41	13 9	41 0	1[illegible]0	8 3	++	++	++++	----	--	++++	+++	+++	N	+++	++
							8 d	45	daily	oral	3 54	14 0	42 5	120		+	+	++++	----	--	+++	+++	+++	N	++	++
							36 d	45	daily	oral	4 06	15 4	46 1	114	7 3	+	N	++	?	?	?	?	?	?	?	N
								+15 units liver extract daily		I M																
3	H C	59	F	P A S C D	5	15 Large treatment for 3 yr	Before				1 55	6 9	18 9	122	4 28	+++	++	++	--	N	N	++	-	N	N	+
							53 da	15	daily	oral	3 59	11 3	34 8	97	10 8	N	+	N	--	N	N	+	-	N	N	N
							5 d	100	daily	oral	3 60	11 3	36 1	100	5 0	N	+	N	--	N	N	+	-	N	N	N
							10 da	100	daily	I V	3 42	10 4	33 1	97	6 95	N	+	N	--	N	N	+	-	N	N	N
							19 da	15	daily	oral		11 2	36 3			N	+	N	--	N	N	+	-	N	N	N
							12 da	15	daily	oral		11 4	36 4		7 2	N	N	N	N	N	N	N	N	N	N	N
								+15 units liver extract daily		I M																
							6 mo after start	15	daily	oral	4 77	12 0	40 5	85		N	N	N	N	N	N	N	N	N	N	N
							10 mo	15	daily	oral	3 79	12 7	39 0	103		N	N	N	N	N	N	N	N	N	N	N
							16 mo	15	daily	oral	4 07	13 4	39 0	96		++	N	++	N	N	N	N	N	N	N	N
							16½ mo	15	daily	oral	4 19	13 4	39 5	94		++	+	+++	--	--	+++	+	N	N	N	+
								+15 units liver extract q 2 wks		I M																
							17 mo	15	daily	oral	3 86	12 7	39 0	101	8 95	++	N	+++	--	-	+++	++	++	N	+++	++
								+ 5 units liver extract weekly		I M																
							17½ mo	folic acid omitted 75 units liver extract weekly		I M	4 24	13 4	42 0	96		++	N	+++	--	-	+++	++	++	N	++++	+++

No.	Initials	Age	Sex	Dx			Time	Dose	Frequency	Route															
1	E B	72	F	P A	9	30	B f				3 59	12 1	38 0	106	6 5	✓	N	✓	✓	✓	✓	✓	N	✓	✓	✓
							2 m	100	m nthly	I M	3 68	12 2	37 5	102		✓	✓	+	✓	✓	✓	✓	✓	✓	✓	✓
							6 m	100	monthly	I M	3 11	11 9	36 0	116		N	✓	++	--	✓	✓	✓	✓	✓	✓	✓
							7 m	1 25	d ly	l	3 39	11 9	35 5	105	7 6	N	N	++	--	✓	✓	✓	✓	✓	✓	✓
							10 mo	15	d ly	o al	3 14	11 7	34 5	110		✓	✓	++	--	✓	✓	--	--	✓	✓	✓
							11 mo	15	d ly	o l	3 40	12 6	37 5	110		+	✓	+++	---	--	✓	---	---	✓	++	+
18	C W	46	F	P A	9	30	Bef				3 77	13 5	40 0	106	6 9	✓	✓	✓	N	✓	✓	✓	✓	✓	✓	✓
							3 mo	100	monthly	I M	4 69	13 3	40 5	86		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							7 m				3 82	12 9	39 0	102		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							9 mo	1 25	d ly	o l		12 3	39 0			N	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							12 mo				3 91	12 6	39 5	99		✓	✓	N	N	✓	✓	✓	✓	✓	✓	✓
19	J L	50	M	P A	5	30	B f e				4 31	13 8	40 5	94		N	✓	N	✓	✓	✓	✓	✓	✓	✓	✓
							3 mo	100	monthly	I M	4 91	14 3	42 0	86		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							7 m	100	mo thly	I M	4 40	14 0	40 0	91		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							10 m	100	m thly	I M	4 27	13 0	39 0	91		N	N	N	✓	✓	✓	✓	✓	✓	✓	✓
0	M G	61	Γ	P A	8	30	B fo e				4 05	13 6	39 5	97	6 1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							4 m	40	m thly	I M	4 07	14 3	42 0	103		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							6 mo	40	m thly	I M	4 38	13 7	40 0	91		N	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							9 mo	1 25	d ly	al	4 33	14 1	4 0	97		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							12 mo	1 25	d ly	ral	4 05	14 4	41 5	103		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
21	C M	71	F	P A	4	30	B fo e				4 46	14 2	44 5	100	9 2	✓	✓	✓	✓	✓	✓	✓	N	✓	✓	✓
							4 m	30	montl ly	I M	4 21	14 5	44 0	105		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							5 m	1 25	da ly	o al						✓	N	✓	✓	✓	✓	✓	✓	✓	✓	✓
							6 m	1 25	d ly	or l	4 25	14 6	46 0	108		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							12 m	1 25	d ly	l	4 77	15 0	46 5	97		✓	✓	✓	✓	N	N	✓	N	✓	✓	✓
22	J S	69	M	P A	8	30	Bef				4 31	14 9	46 0	107	9 2	✓	N	✓	✓	✓	✓	✓	✓	✓	✓	✓
							5½ mo	30	m thly	I M	1 61	7 8	23 0	143		+++	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							6 m	15	d ly	al	2 23	9 1	29 0	130	9 5	++	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
							8 m	15	d ly	l	4 33	13 5	44 0	101		N	✓	✓	✓	✓	✓	N	✓	✓	✓	✓
							8½ mo	15	d ly	l	4 37	14 5	44 0	101	9 4	+	✓	+	–	✓	✓	✓	✓	✓	✓	✓
							9½ mo	15	da ly	o l	4 39	14 3	45 0	103	10 0	+++	✓	+++	----	---	+++	+++	++	+	+++	++++
								+105 un ts l er extract d ly		I M																
							10½ mo	15	d ly	al	4 36	14 7	45 0	103	11 5	+	✓	+++	----	---	++	++	++	✓	++	++
								+75 ts l er e t act we kly		I M																
							11½ m	fol c acid m tted 30 un ts l ver e tract w kly		I M	5 31	15 6	48 5	91	10 5	+	✓	++	----	---	+	++	++	✓	+	++
							11¾ mo	30 u ts l er t act weekly		I M	5 47	16 9	51 5	94		N	N	✓	--	✓	✓	+	+	✓	✓	N

TABLE 1 —*Continued*

Case	Name	Age	Sex	Initial Diagnosis	Duration of Illness before Folic Acid	Previous Treatment with Liver Extract	Folic Acid Therapy: Duration of Therapy or Time in Relation to Start	Dosage: Mg	Dosage: Frequency	Dosage: Route	Blood Findings: Red cell count	Hemoglobin	Hematocrit	MCV	White cell count	Clinical Observations: Weakness	Glossitis	Paresthesias	Vibration sense	Position sense	Incoordination	Knee jerks	Ankle jerks	Babinski's sign	Romberg's sign	Abnormal gait
					years	months					× 10^6	gm/100 cc	%	cubic microns	× 10^3											
11	H H	79	F	P A minimal S C D	6	60	Before				5 06	14 2	45 5	90	10 0	N	N	N	-	N	N	N	N	N	N	N
							3 mo	2 5	daily	oral	15	14 1	45 5	89		N	N	N	-	N	N	N	N	N	N	N
							5 mo	2 5	daily	oral		14 9	46 5			N	N	N	-	N	N	N	N	N	N	N
							11 mo	5	daily	oral	4 76	14 1	45 0	95	8 0	N	N	N	-	N	N	N	N	N	N	N
12	R D	67	M	P A S C D	2	60	Before				4 43	14 6	43 0	97		N	+	+	N	---	N	N	--	N	N	N
							3 mo	1 25	daily	oral	4 78	15 4	46 5	97		N	N	+	--	---	N	N	--	N	+	N
							8 mo	1 25	daily	oral	4 5	15 1	47 0	99		N	+	+	--	---	N	N	--	N	+	N
							1 mo	1 25	daily	oral	4 78	15 5	45 5	95		+	N	++	---	---	-	N	--	N	++	+
13	A T	62	F	P A S C D	5	60	Before				3 73	12 3	39 0	104	7 8	N	N	+	-	N	N	-	-	N	N	N
							3 mo	1 25	daily	oral	3 95	12 5	38 0	96		N	N	N	-	N	N	-	-	N	N	N
							6 mo	1 25	daily	oral	4 46	13 8	42 0	94		N	+	+	--	N	N	-	-	N	N	N
							10 mo	15	daily	oral	4 44	12 9	39 5	89		N	N	+	--	N	N	--	--	N	N	N
							12 mo	15	daily	oral	3 88	12 4	38 0	98		N	N	++	---	N	N	--	--	N	++	N
14	A H	43	F	P A	7	30	Before				4 41	12 5	39 5	90	9 6	N	N	N	-	N	N	N	N	N	N	N
							4 mo	1 5	daily	oral	3 76	12 5	37 0	98		N	N	N	-	N	N	N	N	N	N	N
							7 mo	1 25	daily	oral	4 35	13 2	38 0	87		N	N	N	N	N	N	N	N	N	N	N
							9½ mo	15	daily	oral		12 8	37 5			+	N	+	-	-	N	+	N	N	+	N
							11 mo	15	daily	oral	3 59	12 3	36 0	100		+	N	++	--	-	N	++	+	N	++	+
							12 mo	15	daily	oral	4 87	12 5	38 0	78		+++	N	++	---	--	N	+++	+	N	+++	++
								+30 units liver extract weekly		I M																
15	S S	62	F	P A	1	30	Before				4 89	13 8	42 5	87		N	N	N	N	N	N	N	N	N	N	N
							2 mo	1 25	daily	oral	4 85	14 4	42 5	88		N	N	N	N	N	N	N	N	N	N	N
							7 mo	1 25	daily	oral	4 64	13 2	40 0	86		N	N	N	-	N	N	N	N	N	N	N
							9½ mo	15	daily	oral	4 35	13 8	42 0	96		N	N	N	--	N	N	--	--	N	N	N
							1 mo	15	daily	oral	4 57	13 2	40 0	88		N	N	N	---	N	N	---	---	N	N	N
16	V S	67	F	P A	3	30	Before				4 75	13 2	42 0	88	6 2	N	N	N	N	N	N	N	N	N	N	N
							3½ mo	1 25	daily	oral	4 92	12 9	40 5	82	6 3	N	N	N	N	N	N	N	N	N	N	N
							8 mo	1 25	daily	oral	4 71	13 1	40 5	86	7 6	N	N	N	N	N	N	N	N	N	N	N

been subdivided according to the maintenance dose of folic acid administered (table 2) An increase in dosage was made in 8 patients during the latter half of the period of observation and specific reference to these changes will be made The hemato-

TABLE 2—Results of Maintenance Therapy with Synthetic Folic Acid

Folic Acid Dosage	No. of Patients	Duration of Therapy	Hematologic Status: No change	Hematologic Status: Persistently higher blood level than with lower dosage	Hematologic Status: Initial higher blood level decreasing after 6 months	Hematologic Status: Relapse	Neurologic Status: No change: No.	Neurologic Status: No change: Patients	Progression or Development of Subacute Combined Degeneration: Probable: No.	Probable: Patient	Probable: Time of onset	Definite: No.	Definite: Patient	Definite: Time of onset
mg		mo									mo			mo
Oral (daily)													#1	11
15.0	5	8–12		#4	#1 #3		2	#5				3	#3	16
					#5 #6			#6					#4	12
10.0	2	12	#8		#7		1	#7	1	#8	11			
5.0	2	1			#9		1	#9				1	#10	12
					#10									
2.5	1	11	#11				1	#11						
1.25	5	9½–11	#16	#12			1	#16	1	#12	12			
(Increased to 15)	(3)	(1½–2)	#14		#13				2	#13	12	1	#14	9½
			#15							#15	7			
Intramuscular injection (monthly) 100	3	7–10			#19		1	#19						
(Changed to oral daily) 1.25 at 9 months	(1)	3			#18		1	#18						
1.25 at 7 months	(1)	5	#1									1	#1	6
15.0 at 10 months	(1)													
	1	9												
40 (Changed to oral daily) 1.25 at 9 months	(1)	3	#20				1	20						
30	2	5–6				#2								
(Changed to oral daily) 1.25 at 5 months	(1)	7	#21				1	#21						
1.0 at 6 months	(1)	6										1	#22	8½
Totals	21		8	2	10	1	10		4			7		

Figures preceded by # are numbers of cases listed in Table 1

logic and clinical data before and during folic acid therapy are presented in tables 1 and 2

Oral maintenance dose of 15 mg daily Five patients (Cases 1 3 4 5 and 6) were given a daily oral maintenance dose of 15 mg of folic acid With the exception of 1 patient (Case 6) who was treated for only eight months these patients remained on the same dosage schedule for twelve months Satisfactory blood levels were ob-

TABLE I ADDITIONAL REMARKS

Case 1 Rapid remission induced Maximum reticulocytosis of 29% on 8th day Blood declined to subnormal levels after 6th month Probable development of mild S C D beginning in 7th month

Case 2 Mild anemia but marked macrocytosis Maximum reticulocytosis of 5 8% on 7th day Slight imrpovement in blood during 28 days of folic acid therapy Normal hemoglobin level but persistent macrocytosis following liver extract therapy No improvement in S C D with folic acid therapy

Case 3 Respon e to folic acid comparable with response to liver extract 5 years previously Maximum reticulocytosis 11 8% on 6th day Suboptimal blood levels not improved with liver extract probably because of complicating disease Evidence of S C D at 16 months Rapid progres ion of S C D in spite of institution of liver extract therapy during continuation of folic acid

Case 4 Blood levels higher and macrocytosis less than when under liver extract therapy S C D beginning in 11th month and progressing rapidly in spite of liver extract therapy while folic acid continued

Case 5 For 8 months blood levels much higher than with liver extract therapy Moderate decrease after 8th month No definite evidence of S C D

Case 6 Blood levels decreased at 8 months No evidence of S C D

Case 7 Blood levels improved initially Subsequently decreased and fluctuated in fashion similar to that observed during liver extract therapy No evidence of S C D

Case 8 Blood levels same as with liver extract therapy Probable development of early S C D

Case 9 For 8 months blood levels much higher than with liver extract Then gradual decrease to initial levels No evidence of S C D

Case 10 Blood levels initially higher than with liver extract subsequent decrease Definite development of S C D

Case 11 Blood levels same as with liver extract No change in S C D

Case 12 Blood levels better than those maintained with liver extract Progression of S C D

Case 13 Blood improved initially but decreased to previous levels in 10th month Definite progression of S C D in 12th month

Case 14 Blood levels unchanged even after increase in folic acid dosage S C D developed at 9½ months Progressed rapidly when folic acid dosage increased to 15 mg Continued progression of S C D after institution of liver extract while folic acid continued

Case 15 Blood unchanged Signs of S C D appeared in 7th month and progressed in spite of increased folic acid dose

Case 16 Blood unchanged No evidence of S C D

Case 17 Decrease in blood levels during period of monthly injections Slight improvement on daily oral do e of folic acid Evidence of S C D appeared in 2nd month and progressed in spite of increased dose of folic acid

Case 18 Temporary decrease in macrocyto is No evidence of S C D in spite of very low dose of folic acid

Case 19 Transient increase in blood levels No e idence of S C D Died of heart failure in 11th month

Case 20 Blood levels comparable ith those maintained with li er extract Patient required 11 months to relapse after omission of liver extract on a previous occasion No S C D

Case 21 Blood levels comparable with those maintained with li er extract therapy Improved with oral folic acid therapy No S C D

Case 22 Hematologic relapse during monthly injections Remission induced ith oral folic acid Rapid onset and progression of S C D hen blood was normal Very gradual and incomplete improvement with liver extract therapy while folic acid continued More rapid impro ement after omission of folic acid

patients (including Case 22 previously described) the clinical and hematologic aspects of the disease had been well controlled with liver extract for from one to nineteen years prior to the substitution of folic acid therapy In 2 patients (Cases 1 and 3) folic acid induced remissions preceded maintenance therapy This group has

been subdivided according to the maintenance dose of folic acid administered (table 2) An increase in dosage was made in 8 patients during the latter half of the period of observation and specific reference to these changes will be made The hemato-

TABLE 2—Results of Maintenance Therapy with Synthetic Folic Acid

Folic Acid Dosage	No. of Patients	Duration of Therapy	Hematologic Status: No change	Persistently higher blood level than with liver extract	Initial higher blood level decreasing after 6 months	Relapse	Neurologic Status: No change: No.	No change: Patient	Progression or Development of Subacute Combined Degeneration: Probable: No.	Probable: Patients	Probable: Time of onset	Definite: No.	Definite: Patients	Definite: Time of onset
mg		mo									mo			mo
Oral (daily) 15.0	5	8 17		#4	#1 #3 #5 #6		2	#5 #6				3	#1 #3 #4	11 16 12
10.0	2	12	#8		#7		1	#7	1	#8	11			
5.0	2	12			#9 #10		1	#9				1	#10	12
2.5	1	11	#11				1	#11						
1.25	5	9½ 11	#16	#12			1	#16	1	#12	12			
(Increased to 15)	(3)	(1½ ½)	#14 #15		#13				2	#13 #15	12 7	1	#14	9½
Intramuscular injection (monthly) 100	3	7 10			#19		1	#19						
(Changed to oral daily)														
1.25 at 9 months	(1)	3			#18		1	#18						
1.25 at 7 months	(1)	5	#17									1	#17	6
15.0 at 10 months	(1)													
	1	9												
40 (Changed to oral daily)														
1.25 at 9 months	(1)	3	#20				1	#20						
30	2	5–6				#22								
(Changed to oral daily)														
1.25 at 5 months	(1)	7	#21				1	#21						
15.0 at 6 months	(1)	6										1	#22	8½
Total	21		8	2	10	1	10		4			7		

Figures preceded by # are numbers of cases listed in Table 1

logic and clinical data before and during folic acid therapy are presented in tables 1 and 2

Oral maintenance dose of 15 mg daily Five patients (Cases 1 3 4 5 and 6) were given a daily oral maintenance dose of 15 mg of folic acid With the exception of 1 patient (Case 6) who was treated for only eight months these patients remained on the same dosage schedule for twelve months Satisfactory blood levels were ob-

served early in the course of treatment in all 5 patients and 1 patient (Case 4) consistently maintained higher levels with folic acid than he did with liver extract A decline in the red cell count and hemoglobin to suboptimal levels began after the fourth and sixth month respectively in 2 patients (Cases 1 and 3) in whom remission had been induced with folic acid A similar drop in blood levels was not observed in the other 2 patients (Cases 5 and 6) until the eighth month In 1 patient (Case 3) a significant macrocytosis developed concomitantly with the decline in blood levels

All 5 patients felt extremely well and were asymptomatic during the first eight to eleven months of treatment In the twelfth month of therapy definite signs of neurologic disease appeared in Case 1 (see case report) In Case 3 in the sixteenth month of folic acid therapy and twelve months after the completion of a supplementary course of liver extract therapy the patient developed the symptoms and signs of neurologic disease (see case report)

In Case 4 there had been mild but quiescent subacute combined degeneration for at least eight years and there was no change in this condition during the first ten months of folic acid therapy Beginning in the eleventh month, however the patient developed paresthesias of the hands and feet and weakness and stiffness of the legs His family stated that he became very irritable and occasionally wept because of anger By the twelfth month these symptoms were worse and there was unsteadiness of gait and difficulty in walking Knee jerks were increased and vibration sense was decreased at and below the iliac crests Liver extract was started in addition to continuation of folic acid but in spite of the injection of 260 units in the next three weeks there was rapid progression of neural disease By the end of this time the patient was unable to walk because of weakness of the legs and unsteadiness of gait He complained of warmness of the hands and feet He showed increased muscle tone in the legs markedly hyperactive knee jerks absent left ankle jerk and decreased right ankle jerk a positive Babinski sign on the left and normal plantar response on the right Position and motion sense of the toes was poor The heel to knee test was poorly performed Vibration sense was absent below the iliac crest Romberg s sign was markedly positive Folic acid was discontinued and the patient was hospitalized A lumbar puncture yielded normal fluid

This patient developed progression of subacute combined degeneration during folic acid therapy which proceeded rapidly in spite of parenteral injection of large amounts of liver

Oral maintenance dose of 10 mg daily Two patients (Cases 7 and 8) were given 10 mg of folic acid daily for a period of twelve months Case 8 maintained constant blood levels Case 7 showed an initial improvement in red cell count and hemoglobin but a persistent macrocytosis and the blood subsequently returned to levels essentially similar to those observed during liver extract treatment

In Case 8 progressive diminution of vibration sense in the lower extremities and fingers appeared during the eleventh month and in the twelfth month Romberg s sign became positive

Oral maintenance dose of 5 mg daily Two patients (Cases 9 and 10) were maintained on 5 mg of oral folic acid daily for twelve months In both patients there was an

initial increase in the red cells and hemoglobin and a decrease in macrocytosis but in the eleventh and twelfth months the red cell count and hemoglobin returned to pre folic acid values and in Case 9 there was recurrence of macrocytosis

Subjective improvement and an increase in appetite were noted by each patient with beginning of folic acid therapy and these continued throughout the period of observation Case 9 remained asymptomatic and maintained a normal neurologic system during the period of observation Paresthesias and diminution in vibration sense appeared in Case 10 in the eleventh month By the twelfth month there was marked diminution of vibration sense in the toes and ankles a positive Romberg s sign a slightly unsteady gait hypoactive knee and ankle jerks and a positive Babinski s sign

Oral maintenance dose of 2 5 mg daily Only one patient (Case 11) was given a daily oral dose of 2 5 mg of folic acid This patient maintained as high blood levels throughout the eleven months of treatment as she had previously done with large doses of liver extract She remained asymptomatic and showed no change in her neurologic signs of minimal subacute combined degeneration of the spinal cord

Oral maintenance dose of 1 25 mg daily Five patients (Cases 12–16) were given daily doses of 1 25 mg of folic acid by mouth This dosage was increased to 15 mg daily in 3 patients (Cases 13 14 and 15) in approximately the tenth month of treatment With the exception of Case 16 who was observed for only eight months the members of this group remained on folic acid for eleven twelve or thirteen months The blood picture of 3 of these patients was comparable to that previously maintained by them on liver extract In Case 12 the blood levels were better and in Case 13 there was a temporary increase in red cells and hemoglobin and a decrease in macrocytosis which persisted for eight months and then returned to the levels previously maintained with liver extract

Two of these patients (Cases 12 and 13) had subacute combined degeneration of the spinal cord at the time folic acid therapy was started but in both the disease had remained stationary for several years under liver extract therapy In Case 12 progression of neural disease was shown during the twelfth month of folic acid therapy Weakness and stiffness of the legs numbness of the feet unsteadiness in gait and swaying on Romberg s test became definitely severer at this time and led to institution of liver extract therapy In Case 13 the patient complained of a *squeezing sensation in the feet and calves and there was a definite decrease in* vibration sense below the level of the iliac crests and a markedly positive Romberg s sign during the twelfth month of therapy

After nine and one half months of folic acid treatment the patient in Case 14 showed development of subacute combined degeneration The dosage of folic acid was increased to 15 mg daily by mouth for the next six weeks but the signs of neurologic disease progressed even more rapidly By the eleventh month of maintenance therapy the patient exhibited weakness of the entire body and a numbness and tingling of the hands and feet Knee and ankle jerks were extremely hyperactive vibration sense was markedly impaired below the iliac crests and was entirely absent in the left leg An injection of 75 units of liver extract was given which produced only minimal improvement in one week s time Folic acid was

served early in the course of treatment in all 5 patients, and 1 patient (Case 4) consistently maintained higher levels with folic acid than he did with liver extract A decline in the red cell count and hemoglobin to suboptimal levels began after the fourth and sixth month, respectively in 2 patients (Cases 1 and 3) in whom remission had been induced with folic acid A similar drop in blood levels was not observed in the other 2 patients (Cases 5 and 6) until the eighth month In 1 patient (Case 3) a significant macrocytosis developed concomitantly with the decline in blood levels

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Oral maintenance dose of 5 mg daily Two patients (Cases 9 and 10) were maintained on 5 mg of oral folic acid daily for twelve months In both patients there was an

daily oral dose of 1 25 mg of folic acid, but no change in the blood picture in Case 20 followed such substitution

In Cases 20 and 21 the patients remained asymptomatic and showed no evidence of neurologic damage throughout the period of folic acid therapy As described previously the patient in Case 22 exhibited an explosive development of spinal cord disease during the eighth month of observation despite a normal blood and bone marrow

Discussion

HEMATOLOGIC ASPECTS

Induction of Remission Orally administered folic acid induced hematologic remissions that were comparable in rate and completeness with those that would have been predicted in response to liver extract therapy Reticulocyte responses to folic acid were less marked and subjective improvement was not so sudden in onset or so marked as usually follows exhibition of liver extract Glossitis was not so rapidly or so completely relieved and tended to recur in mild form during folic acid therapy

Maintenance Therapy When folic acid was substituted for liver extract the majority of the patients showed a transitory but significant increase in blood levels and a decrease in mean corpuscular volume This initial but unsustained improvement may have been due to the combined effect of folic acid and liver extract since the latter is stored in the body and may continue to exert its hematopoietic effect for many months after its injection [4, 6]

These observations might be taken to suggest that the patients had not received optimal doses of liver extract before substitution of folic acid but most of them had been given large doses of liver extract without improvement in the blood picture It seems unlikely that the improvement in response to folic acid occurred spontaneously as a result of a nonspecific effect of folic acid or as a manifestation of the cyclic variations observed in pernicious anemia [6]

With continuation of folic acid therapy alone there was usually a gradual fall in blood levels and the appearance of slight macrocytosis This decrease usually became apparent after six to eight months of therapy but in several cases it did not appear until the twelfth or fourteenth month of therapy In most cases the blood returned to levels similar to those previously maintained with liver extract alone but in several patients moderately anemic levels with definite macrocytosis appeared

These findings may indicate that some factor in addition to folic acid is necessary for maintenance of a completely normal blood picture and they suggest the possibility that a combination of orally administered folic acid and parenterally injected liver extract may be more effective than either substance alone

Dosage All patients receiving folic acid in daily oral doses maintained blood levels at least comparable with those previously achieved with liver extract During the period of our observations a daily oral dose of 1 25 mg of folic acid was as effective as a 15 mg dose in controlling hematologic manifestations The patients who received the larger doses more frequently showed an initial improvement in

blood levels (table 2) but these higher levels were not better maintained with the larger than with the smaller doses

The injection of folic acid at monthly intervals did not maintain normal blood values The rate of relapse in a patient who received injections of 30 mg once a month indicated that this amount of folic acid had no effect in maintaining re mission The simplicity and greater effectiveness of orally administered folic acid indicates that the oral route is the rational method of employing the substance

NEUROLOGIC ASPECTS

Folic acid does not prevent the development or progression of subacute combined degeneration of the spinal cord in patients with pernicious anemia In this series of 22 patients treated with folic acid 7 (Cases 1 8 10 14 15 17 and 22) developed neurologic relapse 4 (Cases 3 4 12 and 13) showed progression of subacute com bined degeneration, and 1 previously untreated case of severe subacute combined degeneration (Case 2) failed to improve during twenty-eight days of folic acid therapy The blood levels were maintained within the range of normality in all but 1 (Case 1) of the patients who developed neurologic relapse emphasizing the dissociation of the hematologic and neurologic aspects of the disease and indi cating that the effective hematopoietic factor is distinct from the factor necessary for the maintenance of normal central nervous system function

Neurologic relapse occurred with considerable suddenness and progressed with great rapidity in several patients This rapidity of progression was considerably greater than is usually observed in patients with untreated pernicious anemia Apparently this also was true in similar cases reported by Meyer [7] Heinle and Welch[8] and Vilter et al [9]

We have continued daily oral administration of 15 mg of folic acid after insti tuting liver extract treatment in 5 patients (Cases 3 4 14 17 and 22) in whom neurologic relapse developed during folic acid treatment The period of observation with combined liver extract and folic acid treatment is still quite brief but there has been progression of neurologic disease in 4 of these cases (Cases 3 4 14 and 17) Although improvement in gait and coordination occurred *slowly in the fifth* patient (Case 22) while folic acid was continued vibration and position sense did not begin to show improvement until folic acid was omitted two months after the beginning of liver extract treatment

Development or progression of subacute combined degeneration appeared to occur with the greatest frequency in patients receiving large daily doses of folic acid Eight patients were receiving 10 or 15 mg daily by mouth when neurologic signs or symptoms first appeared whereas only 3 developed neurologic disease when the daily dose was 5 mg or less These observations are not conclusive be cause 4 of the cases on the 15 mg dosage had previously received smaller doses These patients might have developed subacute combined degeneration on the lower dosage had it been continued and the apparent precipitation of neurologic relapse when the dosage was increased may have been coincidental We are impressed however by the fact that of 7 patients (Cases 9 10 11 12 18 20 and 21) main tained on a low dosage (5 mg or less a day) for twelve months only 2 (Cases 10 and 12) showed neurologic relapse One of these (Case 12) had had long standing

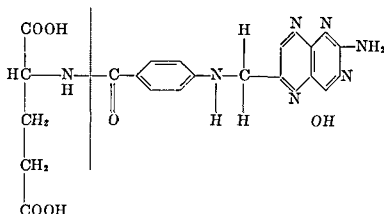
severe subacute combined degeneration and the progression of his disease was minimal In marked contrast of 6 patients treated with 10 or 15 mg of folic acid daily *for twelve months* (*Cases 1 3 4 5 7 and 8*) *neurologic relapse occurred in 4* cases (Cases 1 3 4 and 8) within twelve months of the last injection of liver extract The number of cases in each series is too small and the variation from patient to patient in susceptibility to subacute combined degeneration is too great to allow definite conclusions to be drawn from these observations but it was quite certain that the patients who received large doses of folic acid developed neurologic relapse more frequently than did those who received small doses It was of interest that of 12 reported cases of neurologic relapse occurring during folic acid therapy all received 10 mg or more of folic acid daily [7-10] Heinle and Welch s patient apparently showed acceleration of subacute combined degeneration when the dosage of folic acid was increased to 100 mg daily The neurologic signs apparently progressed even after liver extract was started and did not regress until folic acid was discontinued In contrast to our patients all of the reported cases were in hematologic relapse prior to the induction of folic acid therapy All but 2 of our patients had been under intensive liver extract therapy before substitution of folic acid It may be this difference rather than the larger folic acid dosage that accounts for the earlier development of subacute combined degeneration in the reported cases The fact that the majority of our patients did not develop neurologic relapse until twelve months after the last injection of liver extract may have been due to residual stores of liver extract as a result of previous therapy

The failure of synthetic folic acid to prevent the development or induce the remission of subacute combined degeneration makes it certain that this substance is not the active principle responsible for maintenance of normal nervous system function in pernicious anemia The possibility that folic acid in large dose actually may exert a deleterious effect on the central nervous system is suggested by three observations the apparent greater tendency for the development of subacute combined degeneration in patients who received large doses of folic acid the apparent acceleration of neurologic disease in some patients when folic acid dosag was increased and the persistence or actual progression of neural disease following institution of liver extract therapy so long as folic acid treatment was continued

Theoretically it seems possible that synthetic folic acid in large amounts may contribute to dysfunction of the central nervous system by interfering with the metabolism of l (+) glutamic acid by the central nervous system The observations that suggest this theory are as follows Quastel and Wheatley [11] found that l (+) glutamic acid could be metabolized by nerve tissue and Krebs[12] showed that brain slices could utilize glutamic acid for the synthesis of glutamine Weil Malherbe[13] demonstrated that l (+) glutamic acid was the only amino acid that could be metabolized by central nervous system tissue He observed that d (−) glutamic acid actually interfered with brain metabolism *The essential nature of l (+)* glutamic acid in nerve function was further emphasized by Nachmansohn et al [14] who found that the enzyme system associated with the synthesis of acetylcholine in brain extracts when inactivated by dialysis could be reactivated by the addition of l (+) glutamic acid

These fundamental observations establish the importance of l (+) glutamic acid

in nerve tissue metabolism They also suggest its possible role in the formation of acetylcholine a mediator of nervous impulses Folic acid has the following structural formula



As indicated by the broken line glutamic acid is one of the constituents of the folic acid molecule and its position in the molecule suggests that it may be able to enter into competition with l (+) glutamic acid in tissue metabolism and interfere with nerve metabolism and possibly with the formation of acetylcholine and the transmission of nervous impulses If this interference does occur it explains the greater frequency of neurologic relapse in patients receiving large doses of folic acid and the progression of neurologic disease in folic acid treated patients after the institution of liver extract therapy

That folic acid is connected in some way with acetylcholine metabolism in clinical cases of pernicious anemia is indicated by Davis [15] who reports that there is a marked *increase* in the serum acetylcholine of patients with untreated pernicious anemia and that the administration of folic acid liver extract or ventriculin produces a *decrease* in blood acetylcholine concentration

We are now investigating the possibility that folic acid may actually contribute to dysfunction of the central nervous system by interfering with l (+) glutamic acid metabolism

In view of recent reports that l (+) glutamic acid may be effective in the treatment of feeble mindedness[16] and the possibility that folic acid may interfere with l (+) glutamic acid metabolism it is of interest that 2 of our patients (Cases 3 and 4) who developed neural relapses were noted by their families to have had mental aberrations

Whether or not synthetic folic acid in large doses actually interferes with central nervous system function is still to be determined Clinical evidence proves beyond question that it is not effective in the treatment or prevention of subacute combined degeneration of the spinal cord For this reason it is our opinion that no patient with pernicious anemia should be treated with folic acid alone

Summary and Conclusions

1 Twenty one patients with pernicious anemia were maintained on synthetic folic acid (pteroylglutamic acid) therapy alone for periods ranging from eight to

seventeen months Satisfactory blood levels were maintained in all cases receiving daily oral doses of 1 25 to 15 0 mg Severe hematologic relapse occurred within six months in a case treated with monthly injections of 30 mg

2 Synthetic folic acid in oral doses of 15 mg daily induced satisfactory hema topoietic responses in 3 patients with pernicious anemia in severe relapse but only slight hematopoietic response in a fourth patient with mild pernicious anemia but severe subacute combined degeneration of the spinal cord

3 Ten patients showed a significant improvement in blood values for a few months after substitution of folic acid for liver extract With one exception these subsided after six or more months to pre folic acid levels comparable with those previously maintained with liver extract alone

4 These observations suggest that a combination of orally administered folic acid and parenterally injected liver extract may maintain a better hematologic status than either substance alone

5 A previously untreated patient with severe subacute combined degeneration of the spinal cord failed to show improvement in neural disease during twenty eight days of folic acid therapy

6 Eleven patients developed or showed progression of subacute combined degeneration of the spinal cord during folic acid treatment Neurologic disease developed in most of these patients when the peripheral blood was normal

7 One patient showed an extremely explosive onset and rapid progression of neural disease The progression of the disease was rapid in 3 other cases

8 The institution of liver extract therapy in adddition to folic acid in 5 patients who developed subacute combined degeneration during folic acid maintenance therapy failed to prevent progression of the disease in 4 cases and only partially arrested the disease in the fifth in which improvement occurred more rapidly when folic acid was discontinued

9 Subacute combined degeneration occurred with greater frequency in patients on large daily doses of folic acid than it did in patients with small or intermittent doses

10 The possibility is discussed that folic acid in large daily dos s may actually precipitate or aggravate neurologic disease

11 It is suggest d that folic acid may interfere with the metabolism of l(+) glutamic acid in the central nervous system and possibly disturb the formation or function of acetylcholine

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BLOOD RESPONSE AND NITROGEN BALANCE FOLLOWING LIVER EXTRACT

By Randolph West M D

ONE PATIENT with addisonian pernicious anemia and one with sprue with normal gastric acidity and without diarrhea were studied (Unit history number 432154 and 486382 respectively)

The patients were in the metabolism ward on weighed diets Urinalyses were done on 24 hour specimens stool samples were pooled for several days and aver

Table 1

Sprue

	Day		
	1	7	14
N balance		−11 6	+8 9 Gm per period
RBC	1 0	1 5	2 4 millions
Retics	1 8	49 0	16 0 per cent
Hgb N	26 7	59 2	79 9 Gm total
Plasma N	42 5		42 4 Gm total
Creatine		3 16	1 1 Gm per period

Pernicious Anemia

	Day		
	1	9†	19
N balance		−3 8	+18 4 Gm per period
RBC	2 7	2 6	3 7 millions
Retics	1 9	2 8 7 6	2 6 per cent
RBC N	88		99 Gm total
Plasma N	36		31 Gm total
Creatine		0 781	0 067 Gm per period

Liver extract 150 units parenterally day 2

† Liver extract 45 units parenterally every two days from day 9

aged Plasma volume and red cell volume were done by T 1824 and venous hematocrit serum proteins by the Howe method The main results are given in table 1

In the sprue patient the hematologic response took place while the patient was in negative nitrogen balance in the pernicious anemia case positive N balance and blood response coincided

The plasma N did not increase significantly the rise in hemoglobin N represents a transfer from bone marrow to peripheral circulation

It is of interest that in both cases positive balance was established and creatinuria

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lessened In the pernicious anemia patient this occurred without change in N or caloric intake in the sprue case appetite made increased diet essential to satisfy the patient

Mosenthal[1] in 1918 showed that the forced feeding of a diet rich in meats re stored nitrogen balance in pernicious anemia

The present studies were undertaken to determine whether the positive nitrogen balance appeared before the hematologic response following liver therapy If this had occurred it might indicate that an important site of action of liver extract was on the gut wall This however was not the case and liver extract presumably acts directly on the immature red cells in the marrow cavity

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FURTHER OBSERVATIONS ON THE SPECIFICITY OF THE FOLIC ACID MOLECULE

By Tom D Spies M D Guillermo Garcia Lopez M D Robert E Stone M D * Fernando Milanes M D Robert O Brandenburg M D * and Tomas Aramburu M D

IT HAS been established that pteroylglutamic acid (folic acid) stimulates the development of white blood cells red blood cells and platelets in a variety of animal species and in persons who have certain types of macrocytic anemia Our studies of folic acid have been concerned chiefly with the striking h matologic response which follows its administration to persons with pernicious anemia nutritional macrocytic anemia and tropical sprue in relapse This response has been described in considerable detail [1] and it has been pointed out that it is indis tinguishable from that which follows the administration of refined liver extracts Nevertheless the potency of refined liver extracts is out of all proportion to the amount of folic acid they contain and it is our working hypothesis that the hemopoietic factor in refined liver extracts differs chemically from folic acid per se The study of the synthetic folic acid molecule offers great promise toward de termining something of the nature of blood regeneration Recently we showed[2] that patients who do not show a hematologic response to methyl folic acid will respond to the folic acid molecule Since this study was reported we have investi gated the hemopoietic properties of six additional compounds somewhat related to folic acid in their chemical structure in persons with Addisonian pernicious anemia nutritional macrocytic anemia and tropical sprue in relapse This com munication is concerned with these extended observations on the specificity of the folic acid molecule

Materials and Methods

From a large group of patients 11 patients with macrocytic anemia were se lected for study Four of these were classified as Addisonian pernicious anemia 3 as nutritional macrocytic anemia and 4 as tropical sprue patients In all cases megaloblastic proliferation and defective maturation a red blood cell count of less than 2 5 million and a color index of more than 1 were essential diagnostic cri teria For a differential diagnosis of the type of macrocytic anemia additional criteria were the absence of free hydrochloric acid in the gastric contents even after histamine stimulation in pernicious anemia and the presence of free hydro

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Valuable technical assistance was given by Miss Belle Culver Miss Jane Davis and Mrs Mary B Koch

Williams-Waterman Fellow in Nutrition

chloric acid in nutritional macrocytic anemia and sprue Usually the patients with nutritional macrocytic anemia had diarrhea characterized by loose dark stools The diarrhea present in the patients with sprue was characterized by large, liquid to semisolid foul smelling stools varying in color from whitish yellow to yellowish green A diagnosis of sprue was not made in the absence of steatorrhea The glucose tolerance curve tended to be flat in both nutritional macrocytic anemia and sprue and loss of weight which usually had occurred in both, tended to be greater in sprue than it was in nutritional macrocytic anemia A history of subsistence on an inadequate diet over a period of years was given by all of the patients with sprue and nutritional macrocytic anemia

All the patients were admitted to the hospital where thorough medical and dietary histories were obtained and a complete physical examination was made Rigid dietary control was instituted on admission and continued throughout the duration of the study Meat meat products fish and poultry were excluded Only 1 pint of milk 1 hard cooked egg and 3 level teaspoons of butter were allowed daily Raw vegetables were excluded and all other vegetables were served over cooked All other foods were allowed in any amount desired

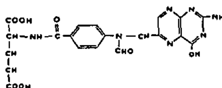


FIG 1 FORMYL PTEROYL GLUTAMIC ACID (FORMYL FOLIC ACID)

For both the white cell and erythrocyte counts certified Trenner pipets were used The hemoglobin content of the blood was determined in grams by means of a Leitz or an Evelyn colorimeter The reticulocytes were counted in wet preparations by the use of a modified brilliant cresyl blue solution of Dameshek In all cases permanent fixed preparations of blood smears were made just prior to treatment and once or twice a week thereafter cell volumes were determined on oxalated venous blood by means of Wintrobe hematocrit tubes Prior to treatment bone marrow was obtained and again at the peak of reticulocytosis and still another specimen was obtained when the reticulocyte count returned to normal Differential counts were made on preparations stained with both supravital and Wright Giemsa stains

After baseline determinations were completed 20 mg of the Mg salt of formyl pteroyl glutamic acid (see fig 1) was administered orally to 1 patient with pernicious anemia and to 1 patient with nutritional macrocytic anemia for ten days Twenty mg of the Mg salt of formyl pteroic acid (S lactis factor) (see fig 2) was administered orally to 1 patient with pernicious anemia for ten days Twenty mg of N-(4-(4-quinazoline) amino) benzoyl) glutamic acid (see fig 3) was given orally to 1 patient with pernicious anemia for ten days Then the dose was increased to 50 mg daily for five days Twenty mg of pteroyl aspartic acid (see fig 4) was given orally to 1 patient with tropical sprue for ten days and 40

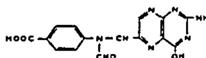


Fig 2 Formyl Pteroic Acid (S lactis Factor)

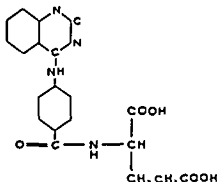


Fig 3 N-(4-(4-Quinazoline)amino)benzoyl)-Glutamic Acid

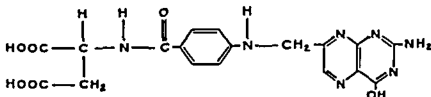


Fig 4 Pteroyl Aspartic Acid

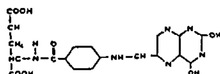


Fig 5 Oxypteroyl Glutamic Acid (Oxyfolic Acid)

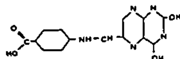


Fig 6 Oxypteroic Acid

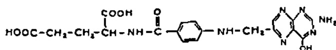


Fig 7 Liver L Casei Factor

N [4 {[(2 amino-4 hydroxy-6 pteridyl)methyl]amino}benzoyl] glutamic acid

mg was given daily for an additional ten days Another patient with tropical sprue was given 20 mg orally for ten days Twenty mg of oxyfolic acid (see fig 5) was

given daily by mouth to one patient with nutritional macrocytic anemia and to 1 patient with pernicious anemia for ten days Twenty mg of oxypteroic acid* (see fig 6) was given daily by mouth to 2 patients with tropical sprue If, within ten days reticulocytosis had not occurred 10 mg of folic acid (see fig 7) was administered until the blood values reached satisfactory levels

Results

Response to Mg Salt of Formyl Pteroyl Glutamic Acid Following the administration of this material to the patient with pernicious anemia, the reticulocytes began to rise on the seventh day and reached a peak of 9 6 per cent eleven days after its administration was initiated This was followed by a slight increase in red blood

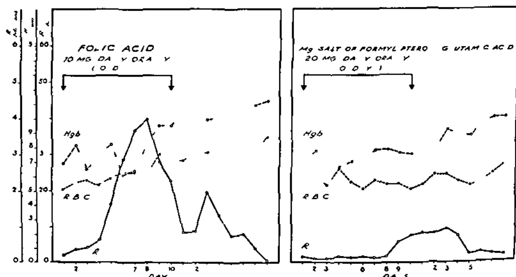


Fig 8 Comparative response to folic acid and Mg salt of formyl pteroyl glutamic acid in a patient with addisonian pernicious anemia

cells hemoglobin white blood cells and platelets The response however was poor compared to his response to the administration of folic acid per se during another comparable relapse of the disease when his reticulocytes began to rise on the fourth day of therapy and reached a peak of 39 8 per cent on the eighth day (see fig 8) By the end of ten days there was an increase in white blood cells and platelets The response of the patient with nutritional macrocytic anemia to Mg salt of formyl pteroyl glutamic acid was slightly greater but it was not of the magnitude which followed folic acid per se Following the administration of Mg salt of formyl pteroyl glutamic acid the reticulocytes began to rise on the fourth day and reached a peak of 25 4 per cent on the tenth day whereas on folic acid per se

The Mg salt of formyl pteroyl glutamic acid and the Mg salt of formyl pteroic acid were furnished by Dr Y SubbaRow of Lederle Laboratories Inc The N-(4-(4-quinazoline) amino) benzoyl)-glutamic acid the pteroyl aspartic acid the oxyfolic acid and the oxypteroic acid were furnished by Dr Gustav Martin of The National Drug Company Only small quantities of these compounds were available so that in no instance did we have an opportunity to test the effect of massive doses

which was administered during another but comparable relapse of the disease the reticulocytes began to rise on the third day and reached a peak of 60 4 per cent on the eighth day (see fig 9) There was a slight rise in the red blood cells hemoglobin white blood cells and platelets following the administration of the Mg salt of formyl pteroyl glutamic acid but they did not reach satisfactory levels until folic acid therapy was initiated

Response to Mg Salt of Formyl Pteroic Acid (S lactis Factor) The administration of this material in the dosage given did not produce blood regeneration in a patient with pernicious anemia whereas an excellent response followed the administration of folic acid per se

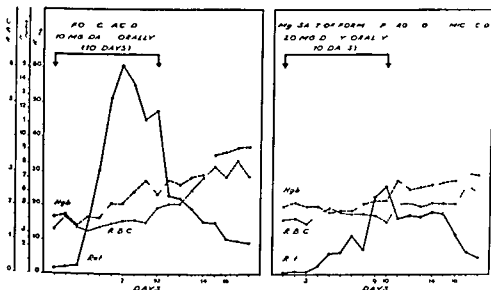


Fig 9 Comparative response to folic acid and Mg salt of formyl pteroyl glutamic acid in a patient with nutritional macrocytic anemia

Response to N-(4-(4-Quinazoline) amino) benzoyl)-Glutamic Acid Neither the patient with pernicious anemia nor the one with nutritional macrocytic anemia had any hematologic response to this material at the dosage level used On subsequent therapy with folic acid an excellent response was observed

Response to Pteroyl Aspartic Acid Blood regeneration did not follow the administration of this material at the dosage level given in either of the 2 cases of tropical sprue Both these patients later showed a good response to folic acid

Response to Oxyfolic Acid The administration of this material produced no blood regeneration in the patient with pernicious anemia or in the one with nutritional macrocytic anemia at the dosage level administered Subsequent treatment with folic acid was followed by an excellent response

Response to Oxypteroic Acid At the dosage level used blood regeneration did not follow the administration of this material in two cases of tropical sprue whereas folic acid therapy which was given later produced an excellent response

Summary and Conclusions

Methyl folic acid[2] N-(4 (4 quinazoline) amino) benzoyl)-glutamic acid the Mg salt of formyl pteroyl glutamic acid the Mg salt of formyl pteroic acid, pteroyl aspartic acid oxyfolic acid and oxypteroic acid have been studied as to their effect on blood regeneration in selected cases of Addisonian pernicious anemia nutritional macrocytic anemia and tropical sprue In the amounts administered only the Mg salt of formyl pteroyl glutamic acid was effective in producing reticulocytosis and an increase in red blood cells hemoglobin white blood cells and platelets and it was not as effective per unit of weight as was folic acid per se Presumably this compound is slowly changed into folic acid in the body It is of special interest that the Mg salt of formyl pteroic acid (Streptococcus lactis factor) was negative in producing hemopoiesis These observations show the very great specificity of the folic acid molecule

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A CASE OF PERNICIOUS ANEMIA REQUIRING ENORMOUS AMOUNTS OF LIVER ESPECIALLY BY MOUTH OVER TWENTY YEARS

By ROGER I LEE M D

I STILL believe that there is a place for the occasional case report in medical literature A disease like pernicious anemia with its protean manifestations and wide variations of course presents difficulties in the way of statistical presentation I have used the present case for clinical demonstration a number of times If we both live her case may be continued

At first I used to demonstrate this patient who needed a large amount of liver over a good many years for the treatment of pernicious anemia as a contrast to another patient who needed liver only once a month or so There was I think no possible doubt of the diagnosis in the 2 cases but the contrasting case unfortunately died of an intercurrent disease

REPORT OF CASE

The case which I report is that of a woman who was seen first in March 19 5 She was a school teacher then 39 years of age She had symptoms of fatigue and weakness with irregular fever beginning in 19 3 When she was first seen the platelets were very much increased and a positive diagnosis of pernicious anemia was not made until August 19 5 The feature of her early course was a continued fever She was put on a diet of raw liver but escaped observation by going to another part of the country where liver was discontinued In 19 8 she developed trouble with her legs In 19 9 she presented a fairly typical ataxic paraplegia She was given one half pound of raw liver a day with a great deal of improvement in her ability to walk although she still presented the clinical picture of ataxic paraplegia Since this episode and to the present time she has required a very large amount of liver She has had liver in every available form If she did not have some liver by mouth and tried to depend entirely upon injections of liver slowly she would develop headache backache and some slight difficulty with her bladder When she increased the liver by mouth these symptoms would slowly subside All the time she was taking injections of liver extract usually once a week and sometimes twice During the war years when liver was difficult to obtain this patient had a particularly hard time in securing the liver to be taken by mouth She did very well on half a pound of beef liver (she could not take pig's liver) six days a week That would represent a yearly intake of 150 pounds of liver by mouth This woman weighed only 100 pounds on the average sometimes less and sometimes more Consequently she ate her weight in liver in a year and this was in addition to the injections of liver In the twenty odd years which she has been under treatment she has taken by mouth certainly over a ton of raw liver In addition we have given her iron all forms of liver substitutes by mouth and every form of liver extract None has had the same effect as the raw liver by mouth We have given her vitamins all without any appreciable effect We have also given her folic acid and this too in addition to the injection of liver and what liver she can take by mouth We have no final opinion on the effect of folic acid yet but it does seem to be beneficial During all this time her hemoglobin has been running from 84 to 94 per cent and her red cell count four and a half to five million

A very curious feature has been that she would develop headache back pain and some increased disturbance of the bladder and in walking long before she showed any evidence of disturbance in her blood Gradually the blood would show evidences of a slight deterioration but this was never very marked The improvement of symptoms would anticipate by a month or two the improvement of her blood These down dips in her symptoms were usually initiated by an intercurrent infection or some complication which made it difficult for her to get the additional liver to take by mouth

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Summary and Conclusions

Methyl folic acid N-(4 (4-quinazoline) amino) benzoyl)-glutamic acid the Mg salt of formyl pteroyl glutamic acid the Mg salt of formyl pteroic acid, pteroyl aspartic acid oxyfolic acid and oxypteroic acid have been studied as to their effect on blood regeneration in selected cases of Addisonian pernicious anemia nutritional macrocytic anemia and tropical sprue In the amounts administered only the Mg salt of formyl pteroyl glutamic acid was effective in producing reticulocytosis and an increase in red blood cells hemoglobin white blood cells and platelets and it was not as effective per unit of weight as was folic acid per se Presumably this compound is slowly changed into folic acid in the body It is of special interest that the Mg salt of formyl pteroic acid (Streptococcus lactis factor) was negative in producing hemopoiesis These observations show the very great specificity of the folic acid molecule

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9

PERNICIOUS ANEMIA NUTRITIONAL MACROCYTIC ANEMIA A TROPICAL SPRUE

A Discussion

By Lucy Wills M A M B B S M R C S L R C P

THE BRILLIANT work of Minot and Murphy (1926 and 1927) on the curative action of liver in Addisonian pernicious anemia and the subsequent work of Castle and his colleagues (1929 1930 1931 1936) on the mechanism of the formation of the liver principle opened up a vast field of research into the nature and the mode of action of this principle in pernicious anemia It was a natural development to extend this field to include a study of other macrocytic megaloblastic anemias and that an explanation of the etiology of all these should be sought on the basis of Castle s theory of an extrinsic intrinsic factor reaction leading to the formation of the liver principle Much experimental and clinical work was carried out on these lines but the concentration on this one aspect of these diseases and recently on the effect of folic acid in the same anemias has by thus limiting the field led to a neglect of the study of the general pathology and the natural history of these conditions and perhaps to a too limited view of their etiology In pernicious anemia nutritional macrocytic anemia and tropical sprue to limit the discussion to the three principle macrocytic anemias anemia is only one aspect of each disease complex and the fundamental differences between the three disease entities have been overlooked in the light of the spectacular success of treatment with liver extracts and folic acid It was attractive to fit these three diseases or rather the anemia in each case into the framework of Castle s theory according to which all three are due ultimately to a deficiency of the liver principle Pernicious anemia thus would arise through a deficiency of the liver principle due to an absence from the stomach of the intrinsic factor nutritional anemia to the same anemia arising from a lack of the extrinsic factor in the diet and the macrocytic anemia of sprue to a failure of absorption of the liver principle in some cases associated with a reduction or absence of the intrinsic factor Even had such an explanation in its simple form withstood the test of clinical trials and experimental work it would not have explained the etiology of the pathologic change in the gastric mucosa in pernicious anemia to which the absence of the intrinsic factor is attributed nor that of the functional changes in the intestinal mucosa which lead to the failure of absorption in sprue This is not to question the validity and applicability of Castle s work his experimental results belong to the group of obstinate facts that have to be reckoned with but the simple explanation of the causes of the postulated deficiency in the liver principle in these diseases must be revised in view of certain experimental and clinical findings Undoubted cases of macrocytic anemia due to a deficiency of the extrinsic factor do exist and respond to treatment with this factor (Moore et al 1944 Watson and Castle 1946) On the other hand

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Anyone who has studied pernicious anemia appreciates the wide variation in the spontaneous course of the disease In this case it is to be noted that fever was an early symptom and that this was by no means slight I regard that as an indication of the intensity of the condition The contrasting case which I used to show with this patient at no time had fever Her condition seemed to be of a gentler kind The patient in this contrasting case in spite of the small amount of liver that she took due to the fact that she felt well never developed the lesions of pernicious anemia in the central nervous system I think it has long been recognized that the more intense cases of pernicious anemia are more likely to have lesions of the central nervous system although it is well known that all long standing cases of pernicious anemia have at autopsy actual lesions in the central nervous system Our patient developed symptoms of the central nervous system early At one time there was a great deal of discussion as to the efficacy of liver therapy in lesions of the central nervous system This case would seem to indicate that liver therapy does not cause complete regression of the lesions of the central nervous system but it keeps them under control

At no time during these twenty years, has this patient had an enlarged liver enlarged spleen hypertension or particularly abnormal blood chemistry although the non protein nitrogen was apt to be in the high 30 s and the uric acid was apt to be around four

This case indicates several things to me

1 The variation in the intensity of the disease of pernicious anemia

2 That involvement of the central nervous system is apt to indicate a disease that may be difficult to treat

3 That some cases of pernicious anemia require relatively enormous amounts of liver therapy and that successful therapy may demand even in these days a combination of oral as well as intramuscular liver

4 Finally this case illustrates the fact that some cases of pernicious anemia present real problems in treatment and cannot be treated by any rule of thumb method

mia of pregnancy in India (Balfour 1927 McSweeney 1927 Wills and Mehta, 1930) it was later reported from British Guiana (Giglioli 1934) East and West Africa, (Trowell 1943) and probably from Puerto Rico for though Castle and co-workers (1935) have described the cases of macrocytic anemia they studied there as sprue it is probable that British workers would consider some at least of their cases as nutritional macrocytic anemia Rodiguez Molina (1939) has described typical cases of nutritional macrocytic anemia from the same island A similar anemia which will be discussed lat r has been reported from Macedonia by Fairley and his colleagues (Fairley et al 1938 Foy and Kondi 1939) Sporadic cases particularly among pregnant women have been reported by many workers from Europe and America but the relation of these cases to the endemic diseases is uncertain Tropical sprue as its name implies occurs mainly in the tropics It was first describ d in 1759 by Hillary in the West Indies and then rediscovered in the Far East (Manson 1879-80) and has since been recognized in many parts of the world Further it has long been observed that certain districts and even houses in endemic areas have a high sprue incidence (Leishman 1945 Keele, 1946)

Seasonal Incidence

Pernicious anemia is not a seasonal disease The highest incidence of nutritional macrocytic anemia in Bombay is during the winter months and the lowest during the monsoon (Balfour 1927) This variation may be related to humidity tempera ture or changes in the small additions to the diet which are of such importance in very deficient and monotonous diets Marriage customs may also influence the incidence as they give rise to a seasonal variation in the number of pregnant women in the community and thus to the number of cases of nutritional macrocytic anemia as this is very prevalent among such women Hare (1946) reports that in Assam the maximal incidence of anemia including macrocytic anemia in pregnant women is in the third quarter of the year which is the rainy season there At this time fresh green vegetables are not available and the home pounded rice normally eaten is replaced by milled rice Napier (1941) in Calcutta found that the highest incidence in pregnant women occurred in the second half of the year in which owing to the hot weather and monsoon less fresh vegetables and fish are taken The onset of diarrhea in sprue is often associated with the hot weather, when in India the barometric pressure is lowest but new cases arise at all times of the year

Populations Affected

A consideration of the racial and social groups who suffer from these three dis eases is of considerable interest as they vary widely Pernicious anemia appears to be par excellence a disease of people of European descent particularly of the Nordic races and is rare in Asiatic or Negro peoples (Friedlander 1934) Within the racial group affected the disease is no respecter of persons occurring in men and women of all social strata but showing a marked familial incidence The individuals affected are characterized by or give a history in other members of the family of a fair complexion and hair and light colored eyes The other two diseases are how ever class conscious Endemic nutritional macrocytic anemia occurs in certain ill

in many areas where nutritional macrocytic anemia is endemic it has been shown that the anemia does not respond to treatment with purified liver extracts known *to be active curatively in pernicious anemia* even when these are given in doses equivalent to or much larger than those effective in pernicious anemia However in other areas cases have been shown to respond to enormous doses of purified extracts when the question of traces of other factors possibly present in the ex tracts has to be considered (for review of literature see Watson and Castle 1946) Animal experiments have also confirmed the inactivity of highly purified extracts in nutritional macrocytic anemia (Wills et al 1937) and now modern work on the therapeutic activity of folic acid in macrocytic anemias has to be fitted into the picture

A consideration of these findings necessitates a reorientation of our mode of approach to the study of these three diseases which does not imply a neglect of the study of the pharmacologic and physiologic action of the elusive liver factor or of that pleasantly concrete substance folic acid But the approach should be widened to include the natural history of these conditions and a study of their general pathology In these studies certain considerations should be borne in mind It should be remembered that the response of the body to any one factor or de ficiency varies with the past and present environmental condition race family sex climate diet illnesses etc all of which will have determined the physiologic and anatomic state of the body at any one moment of time The presence or absence of one factor may condition the mode of action of another factor starving animals on a rachitogenic diet fail to develop rickets Metabolism proceeds by a series of chain reactions this is beautifully seen in cellular metabolism where the members of the vitamin B complex play such an important role in the chain of oxidation reduction reactions The links in these chains may be broken at many points but the breakdowns thus produced may result in very similar pathologic states It should also be stressed that a single symptom complex such as that of a macrocytic hyperchromic anemia may be a part of many different disease entities Much con fusion has arisen for example by the attempt to bring all nutritional macrocytic anemias into one etiological group or to differentiate types on the basis of the serum bilirubin without due consideration of complicating factors With these considerations in view an attempt will be made to review the natural history and to study *the pathogenesis and interrelationships of these three clinical entities* In each case the discussion will be limited to the classic type of each disease though mention will be made to the large group of ill defined and at present not worked out conditions which have many of the signs and symptoms of the clinical entities under consideration

Geographic Distribution

The distribution of the three diseases varies widely Pernicious anemia is mainly a disease of the temperate zones the highest incidence being in the Nordic coun tries the British Isles Canada and the northern portion of the United States of America Nutritional macrocytic anemia in its endemic *form is broadly speaking* a disease of tropical and subtropical lands Originally described as pernicious ane

the atypical cases which may perhaps be the clue to the interrelationship of this group of clinical entities let us consider typical examples of each disease and compare them one with another

In pernicious anemia the patient usually presents as a well-covered slightly lemon yellow colored middle aged or old individual of European descent often already showing signs that the nervous system is involved Very different is the picture in nutritional macrocytic anemia The age of the patient varies from the late teens to relative old age in India I never saw a case in a young child but Giglioli (1934) reports 6 cases out of 51 cases below the age of 12 years the youngest being 11 months old The patient is commonly emaciated but in British Guiana Macedonia and Calcutta all areas where this anemia occurs in a population which has a high malaria rate emaciation is not such a marked feature Some degree of edema is common as in all severe anemias but it is sometimes extreme occasionally from associated beri beri or hunger edema As far as a dark skin will permit one to judge the patient is not jaundiced has clear conjunctivae and with the exception of the prisoners of war has no signs of associated disease of the central nervous system In Bombay where the nutritional anemia is extremely common an occasional case may be frankly jaundiced but nearly always this was found to be associated with syphilis or malaria In highly malarious areas such jaundiced cases are frequent The sprue case generally of European descent and frequently a middle aged individual is emaciated but with a distended abdomen often with visible peristalsis the skin is greyish or a dirty yellow color The signs and symptoms of other deficiencies such as tetany or purpuric manifestations may be present

One sign which together with the anemia has been taken to indicate a close relationship between these conditions is diarrhea This may occur in pernicious anemia but is not a very constant finding and is often controlled by hydrochloric acid alone In nutritional macrocytic anemia the occurrence of diarrhea is of considerable interest In certain years in Bombay it was not a common complication though severe cases of nutritional macrocytic anemia occurred in other years it was noted that diarrhea often associated with a typhoid or hectic temperature which led to the isolation of the patient was a very frequent complication No specific organism could be isolated from the stool and the whole syndrome cleared on marmite or a crude liver extract and did not tend to relapse (Wills unpublished) Giglioli (1934) reported diarrhea in only a few cases whereas Napier (1941) reported a significant positive correlation between macrocytic anemia and diarrhea an incidence of 42 per cent in 45 pregnant cases A very similar sequence of events is seen in monkeys rendered anemic by faulty feeding the anemia which is apparently the counterpart of the human condition might develop to an extreme degree without intestinal symptoms appearing or another time it might be associated with severe diarrhea which with the anemia improved immediately as in the human cases on treatment with active preparations of liver or yeast In the animal cases too no specific organism could be isolated from the stools (Wills unpublished) In returned Indian prisoners of war with a high incidence of nutritional macrocytic anemia diarrhea was rarely complained of and was present in only 5 per cent of those requiring hospital treatment (Walters et al 1947) In sprue

nourished populations, particularly among vegetarians (Taylor and Chhutani 1945 Walters 1947) the poverty stricken poorer inhabitants of Indian cities (Wills and Mehta 1930 Mudaliar 1932 Napier 1941) the East Indian laborer in Central America (Giglioli 1934) Indian troops on active service (Marriott 1945) or the returned Indian prisoner of war (Walters et al 1947) Pregnant women are especially liable to develop the disease Napier (1941) in Calcutta found that in pregnant women suffering from anemia there is a significant correlation between severe anemia and poverty but that the correlation between macrocytosis and poverty though very suggestive is not statistically significant His results also suggested a correlation between vegetarianism and macrocytic anemia but this finding was partly invalidated by the fact of a high positive correlation between vegetarianism and high economic status in the cases studied

Tropical sprue as originally described by Hillary (1759) in Barbados and as seen in India and the Dutch East Indies is in contrast not a disease of the ill fed native resident but of the relatively well fed European or Anglo Indian resident A distinction must be made here apparently between sprue as originally described and seen typically in India and the Far East, and sprue as seen in Puerto Rico In India as mentioned above the disease affects well fed Europeans in Puerto Rico the cases are reported (Castle et al 1935 Rodriguez Molina 1939) as having subsisted for years on a very deficient diet the intake of good biologic protein being particularly low This is not to imply that in the established syndrome the diet is anything but deficient for in the untreated cases it most certainly is the patients limiting their own diets but the disease originates in well fed individuals In this respect an outbreak of acute sprue that occurred among the Chindits in the Burma campaign is of considerable interest These men picked British troops existed for weeks on a ration that was only meant for use in a short emergency After a very short time a matter of days many of the men developed nausea and vomiting and practically all complete loathing of the ration and in a large proportion of the men the picture of advanced sprue developed in a matter of eight weeks (Keele and Bound 1946) This history differs markedly from the usual one in a case of sprue and further work is necessary to fit these cases into the sprue picture Many other cases were also reported from East Asia Command by these and other workers

Clinical Features

It is not proposed to discuss these in detail but merely to point out the striking differences in the three conditions A long experience of numerous cases of pernicious anemia nutritional macrocytic anemia and tropical sprue as seen in India makes it difficult to consider the three conditions as variants of the same disease entity As already mentioned however it must be borne in mind that the same pathogenic agent produces widely differing clinical pictures in individuals whose genetic makeup varies who live under diverse conditions of climate diet housing etc and who have suffered from different stresses and strains all their lives The disease entities may on the other hand be deceptively similar when one particular symptom for example anemia dominates the picture Disregarding for the moment

color as is seen in pernicious anemia The organs particularly the liver and spleen were enlarged and the heart examined only in one case showed some fatty degeneration

The hemopoietic organs and the blood picture are of particular interest in the three diseases The classic picture of a panhemopoietic dystrophy characterized by a megaloblastic erythropoiesis a similar disturbance in the myeloid series with pathologic macro-myeloid cells and a reduction in number and abnormality in type of the thrombocytes is present in all three conditions and the general opinion is that the changes in the cells in marrow and blood are identical in the three diseases But both in sprue (Mackie and Fairley 1929) and in nutritional macrocytic anemia (Balfour 1927 Mitra 1931 Wills unpublished) examination of the tibia may show an aplastic marrow with a curious gelatinous appearance in the shaft though in other cases the red marrow may extend from end to end of the bone In nutritional macrocytic anemia in monkeys the tibial bone marrow may show similar red and gelatinous changes with a megaloblastic hyperplasia (Wills and Stewart 1935) The detailed picture in the bone marrow revealed by sternal puncture preparations varies from case to case with the severity of the anemia and with complicating factors but the essential pathology is the same and the changes resulting from adequate treatment with liver or folic acid are also the same But there is a remarkable difference in the blood condition which has not been adequately stressed In true pernicious anemia in relapse there is some factor constantly present which causes an increase in the serum bilirubin which gives rise to the characteristic coloring of the skin sclerotics and of the body fat and also to an increased output of urobilin or urobilinogen in the urine and feces Fairley (1941) has also shown the presence of methemalbumen in the plasma which is taken as evidence of intravascular hemolysis In uncomplicated cases of tropical nutritional macrocytic anemia in relatively nonmalarious areas and in uncomplicated cases of sprue the findings are in marked contrast to those in pernicious anemia in relapse and in the hemolytic type of nutritional macrocytic anemia as shown in table 1

Earlier figures lost during the blitz from a larger series of cases of nonhemolytic nutritional macrocytic anemia gave similar findings the mean figure for 50 cases being 0 33 mg per 100 ml Only 1 in 36 cases had urobilin or urobilinogen in excess in the urine In the hemolytic type of nutritional macrocytic anemia seen in Macedonia the serum bilirubin in the cases reported by Fairley and colleagues (1938) was markedly raised the mean figure being approximately double that of the author s series of untreated cases of pernicious anemia (see table 1) In an earlier series of 48 cases of sprue seen in Bombay by Fairley only 3 had bilirubin values above 0 6 mg per 100 ml In a series of cases of sprue seen in Puerto Rico (Castle et al 1932) the icterus index was determined in 89 individuals in 24 it was above 6 units which was almost as great an incidence of a raised value as in the same authors series of cases of pernicious anemia It is difficult to assess these figures as Puerto Rico is a malarious area The difference in the figures for serum bilirubin and urinary urobilin or urobilinogen in the different clinical entities suggests that in untreated pernicious anemia and in the hemolytic type of nutritional macrocytic anemia there is a hemolytic factor which is absent in uncom

which commonly develops insidiously but may develop suddenly sore tongue and diarrhea dominate the clinical picture The type of stool is characteristically bulky greasy frothy and pale, differing from that seen in the diarrhea complicating nutritional macrocytic anemia of men and monkeys where it is usually more watery and neither so pale or so frothy But atypical enteric stools may occur in the acute phases of sprue The sore tongue which also occurs in pernicious anemia and nutritional macrocytic anemia is a far more constant feature in sprue

Nervous lesions other than signs of neuritis are lacking in both nutritional macrocytic anemia and tropical sprue In Bombay in several hundred cases signs or symptoms of subacute combined degeneration were absent and Fairley (1936) reports the same absence in 450 cases of sprue seen by him personally Ashford (1932) in a review of 3 000 cases of sprue does not mention any signs or symptoms of this complication (quoted by Fairley, 1936)

Pathology and Biochemical Findings

Since the introduction of liver therapy the uncomplicated case of pernicious anemia seldom comes to postmortem but such was not the case previously and both the older pathologists and the older literature can give a detailed account of the findings Details of the pathology of nutritional macrocytic anemia are not available owing to the difficulty of obtaining permission for postmortems in such cases It is also regrettable that most of the little material that is available came from cases living in areas where malaria is endemic and which were all examples of so-called hemolytic nutritional macrocytic anemia (Fairley 1938) these cases differ in important respects from uncomplicated ones which for clarity will be referred to as nonhemolytic nutritional macrocytic anemia

At postmortem the body in a case of pernicious anemia is usually that of a well nourished middle aged or elderly man or woman the skin and sclerotics and particularly all the fatty tissues are a bright lemon yellow color and there is an excess of fat in and around the organs In contrast in nonhemolytic nutritional macrocytic anemia and in sprue the body is usually emaciated fat being conspicuously absent from all the organs and the characteristic lemon yellow color of pernicious anemia is also missing all the organs are extremely pale A further feature of these two diseases is the great reduction in the size and weight of the organs especially the heart and liver which is in contrast to the findings in pernicious anemia where the organ weight is not reduced and may be increased Fairley (1930) thinks this decrease in organ weight may be of diagnostic significance At the postmortem of a case of nonhemolytic nutritional macrocytic anemia a male of about 23 years of age the body weight was found to be under 6 stone though he was of average height for an Indian and the heart weight was only 140 grams (Wills unpublished) Mackie and Fairley (1929) report a heart weighing only 90 grams in a case of sprue that came to postmortem In contrast are the findings in the hemolytic type of nutritional macrocytic anemia in two incomplete postmortem examinations on a pregnant woman and on a woman who had just been delivered respectively (Fairley et al 1938) the bodies were relatively well nourished subcutaneous fat was plentiful and of the same bright lemon yellow

In most of the cases with enlarged spleens the liver was also enlarged and there was a definite correlation between a raised Van den Bergh and enlargement of these organs There was also a strong suggestion that there is some association between a positive Wassermann reaction and a macrocytic anemia Giglioli (1934) found the spleen enlarged, generally to below the umbilicus in 94 per cent of his cases of macrocytic anemia and associated in the majority of cases with a raised serum urobilin There was a marked positive correlation between the spleen and parasite rate and the incidence of the anemia which suggests he thinks that chronic malarial infection is a factor of very considerable importance in the etiology of nutritional macrocytic anemia In Macedonia the vast majority of the cases had grossly enlarged spleens

In 2 cases of nutritional macrocytic anemia dying after parturition postmortem examination (Balfour 1927) showed very slightly enlarged spleens which, with the livers gave a positive Prussian blue reaction In the 2 cases of hemolytic nutritional macrocytic anemia in pregnant women examined postmortem by Fairley and colleagues the liver was very enlarged in 1 case with nutmeg changes from heart failure and moderately enlarged in the other both livers showed marked hypertrophy of the reticulo-endothelial cells with swollen Kupffer cells phagocytosis and proliferation in the sinusoidal system Malarial pigment was present in some of the Kupffer cells and hemosiderin in the liver cells particularly in the outer zone of the lobules The spleen was enlarged and hard in both cases (25 and 22 7 ounces respectively) and showed a hyperactive reticulo-endothelial system with some malarial pigment in the cells and a reduction in the lymphoid tissue the hemosiderin was less than in the liver The kidneys gave a negative Prussian blue reaction Mitra (1931) found similar changes in material from cases in Calcutta which were probably hemolytic but in his cases there was a fatty degeneration of the central part of the lobule with extravasation of blood

The other systems of particular interest are the alimentary and nervous systems In all three conditions the tongue may show characteristic changes but these may be absent and are not specific as very similar changes occur in microcytic anemia and in pellagra Abnormalities of the stomach and intestines may be present in all three diseases and are of fundamental importance in pernicious anemia Many workers particularly Castle et al (1935) have compared the gut changes in pernicious anemia and sprue with those seen in pellagra and have stressed the atrophic tongue changes and the diarrhea and the similar effect of treatment on these states in all three conditions The dramatic effect of folic acid on the intestinal symptoms in pernicious anemia nutritional macrocytic anemia and sprue also suggests that the lesions of the alimentary tract are similar in all three conditions But though this may be true of the final state of the fully developed disease the basic pathology would appear to be different in the three entities The classic work of Magnus (1938) and Meulengracht (1939) has shown that the fundamental change in pernicious anemia is an atrophy possibly genetic of the mucosa of the fundus of the stomach and it is this lesion which appears to lead by the production of an abnormal gastric juice to a failure in the supplies of the liver factor or factors necessary for proper hemopoiesis and for the good health of the central nervous

plicated nutritional macrocytic anemia and sprue The postmortem findings though these are scanty in nutritional macrocytic anemia are of interest in this connection

The characteristic deposits of iron found in the liver, spleen, bone marrow and kidneys in pernicious anemia are not considered by most recent workers to be an index of increased hemolysis but rather of an inability of the blood forming organs to deal with the iron liberated by normal blood destruction (Minot and Strauss 1943) But the iron in the kidneys is deposited within the cells of the excreting tubules particularly in the proximal tubules the glomeruli containing no iron pigment Muir and Young (1941) have shown that large amounts of hemosiderin may be deposited in the cells of the tubules as a result of a hemolysis insufficient in degree to cause hemoglobinuria and they suggest that these deposits in the kidney in pernicious anemia are due to such a hemolysis Evidence in support of this view is the presence of methemalbumen in the plasma (Fairley 1941) There are insufficient adequate postmortem studies to compare the hemosiderin deposits

TABLE 1 —*Serum Bilirubin Values*

Group	Sex	No	Mean mg per 100 ml	S D	C V	Range mg per 100 ml
Normal*	M & F	100	0 539 ± 0 0247	0 247	45 9	0 2–1 7
Pernicious anemia in relapse†	M & F	27	1 059 ± 0 1336	0 694	65 47	0 4 2 5
Nutritional macrocytic anemia untreated‡	M & F	42	0 516 ± 0 03 5	0 243	47 1	0 1–1 0
Hemolytic N M A §	F pregnant	37	2 0			0 ,–4 2
Tropical sprue ‖	M & F	20	0 37			

* Vaughan J M and Haslewood G A D Lancet 1 133 1938

† Wills L 27 consecutive cases seen at the Royal Free Hospital (unpublished)

‡ Wills L and Evans B Forty two cases seen in Bombay 1937–8 (unpublished)

§ Fairley N H et al 1938 Consecutive cases of Nutritional Macrocytic Anemia (hemolytic type) in pregnant women seen in Macedonia

‖ Fairley N H 1930 Tropical sprue

S D = Standard deviation C V = Co efficient of variation

in complicated nutritional macrocytic anemia and sprue with those in pernicious anemia The size of the liver and spleen are of some interest in this connection In pernicious anemia both organs are commonly moderately enlarged and show the characteristic deposits of hemosiderin and may show areas of extra medullary blood formation fatty changes are very marked in the liver In nutritional macrocytic anemia the size of the liver and spleen appears to vary with the geographic locality and the malarial infection rate In Bombay particularly in the male cases these organs were rarely palpable and in the 2 postmortems the size of the organs was reduced in one case the liver weighed only 630 grams and the spleen 120 grams both organs gave a positive Prussian blue reaction In some of the female patients both organs were just palpable In Calcutta British Guiana and Macedonia all highly malarious areas the findings differ markedly Napier (1941) using pregnant cases reports a very marked association between splenomegaly and severe macrocytic anemia and in 6 out of 44 cases the spleen was below the navel

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system Jacobson (1939) would correlate the presence of similar hemopoietic properties to those of liver in dessicated stomach and small intestine to the presence of argentaffin cells, which are markedly reduced in pernicious anemia, both in the atrophic gastric mucosa and in the intestine The characteristic changes in the gastric mucosa seen in all true cases of pernicious anemia are absent from the gastric mucosa of monkeys suffering from nutritional macrocytic anemia (Magnus—from a study of our material—unpublished) so presumably they are also absent from the stomach in human nutritional macrocytic anemia The presence of free hydro chloric acid in normal amounts would also suggest that the mucosa is undamaged Suitable material for the study of possible changes in the gut in nutritional macrocytic anemia is not available but from analogy with animal material there is probably a thinning of the gut wall due to the general emaciation and little else in the small intestine Nonspecific ulceration of the large intestine was seen in one tropical case that came to postmortem (Wills unpublished) The importance in *the examination of the intestines of fixation immediately after death is* well il lustrated in the postmortem reports on cases of sprue In 1929 Mackie and Fairley reported changes in the small intestine which they considered begin as an in flammation but pass on to degenerative changes (Fairley 1930) More recent work (Mackie and Fairley 1934) on material fixed immediately after death has failed to reveal any pathologic change except slight congestion of the margins of the val vulae connivents and these authorities consider that the changes in the gut which result in such profound metabolic disturbances are functional and not pathologic Hanes (1942) confirmed this absence of pathologic change except extreme emacia tion in 4 fatal cases of sprue Koppisch (Suarez et al 1947) reported the post mortem findings in 16 cases of sprue in Puerto Rico He found evidence but it may have been a postmortem artifact of chronic gastritis in all but 3 cases and moderate atrophy of the gastric mucosa in half the cases Gastroscopic examination confirmed the presence of atrophy of the gastric mucosa (Rodriguez Olleros 1938 Hernandez Morales 1944) but Rodriguez Olleros considers that this atrophy results from the disease and does not precede it as the atrophy was only found in fully developed cases Hernandez Morales found that after treatment the mucosa in many cases became normal again Koppisch also reported a definite shortening and blunting of the villi of the small intestines with an associated increase in the number of plasma cells in the tunica propria in half the cases examined As in *nutritional macrocytic anemia in monkeys and man inflammation and nonspecific* ulceration were found in the colon of the majority of the cases

The nervous lesions of subacute combined degeneration of the cord so char acteristic of pernicious anemia are rarely if ever seen in true tropical sprue or nutritional macrocytic anemia Peripheral neuritis may occur in all three conditions but is an inconstant finding due to an associated dietary deficiency in the vitamin B complex in the nutritional cases and probably to conditioned deficiency in the case of sprue

Chemical examination of the gastric juice in the three conditions shows a com plete and persistent histamine resistant achlorhydria associated with achylia and absence of the intrinsic factor in true pernicious anemia in nutritional macrocytic

anemia and sprue the gastric acid varies from hyperchlorhydria to hypochlorhydria and achlorhydria the last being more frequent in cases of sprue than in cases of nutritional macrocytic anemia The amount of intrinsic factor present in the gastric juice of such cases is also variable being absent in some cases of sprue (Castle et al 1935) and in certain cases of nutritional macrocytic anemia associated with pellagra (Moore et al 1944) It has not been possible to test the gastric juice of cases of uncomplicated tropical nutritional macrocytic anemia as no cases of true pernicious anemia were available in Bombay for test purposes

Various biochemical tests are now used for the diagnosis of sprue the most important being the fat content of the stool the fat absorption test and blood lipid curves and the oral glucose tolerance test It is doubtful whether any of these can be considered to give specific diagnostic results as similar findings to those usually obtained in sprue cases are also found in other conditions such as multiple vitamin B complex deficiencies but in conjunction with a typical history and clinical picture these tests can confirm the diagnosis In sprue the stools are typically bulky pale and fermenting the color being commonly pale but dark colored stools being not uncommon (Black 1945) The stools contain an excess of fat the greater part of which is split Fat balance experiments show a decreased absorption and though after treatment the diarrhea may be controlled and the percentage of fat in the stools decreased fat absorption is still defective and it may be some considerable time before it improves (Black et al 1946 Davidson et al 1947) In nutritional macrocytic anemia the stools frequently appear normal (Wills unpublished Walters et al 1947) Fairley and colleagues (1938) give figures showing a low fat content with normal ratio of split to unsplit fat Such values would be expected in most of the cases as poverty limits the fat intake A dietetic survey in Bombay among the families of patients with nutritional macrocytic anemia showed that the average daily consumption per adult was 45 Gm of which 20 Gm was animal fat (Wills and Talpade 1930) Cook (1944) and Chandhuri (1944) have described a spruelike condition in the civil population in India associated with macrocytic anemia and diarrhea but the diarrhea was watery and not fatty in both series Walters (1947) and Girdwood (Davidson et al 1947) however describe a steatorrhea in a deficiency syndrome resembling sprue in Indian soldiers In certain of these cases the diarrhea improved with nicotinic acid and in many sulphaguanidine controlled it suggesting an underlying infective condition (Marriott 1945 Chandhuri and Chandhuri 1944)

The typical flat oral glucose curves that occur in sprue are not found in the uncomplicated case of nutritional macrocytic anemia (Fairley et al 1938) but in those cases associated with a spruelike syndrome flat curves are found in a few instances (Chandhuri and Chandhuri 19 4)

Treatment

Since the epoch making discovery of Minot and Murphy of the therapeutic activity of liver in pernicious anemia treatment of this and other allied macrocytic anemias has involved the use of different liver extracts with stomach preparations and in the nutritional cases with various so called sources of Castle s extrinsic

factor But until recently, when the discovery of the hemopoietic activity of folic acid and its conjugated forms at last gave workers a chemically pure active substance no pure substance with similar activity was available the various factors postulated in Castle s theory of the formation of the liver principle and the principle itself having remained elusive This fact makes the interpretation of the activity or inactivity of different so called purified preparations difficult as varying doses mean varying amounts of substances other than the liver principle which when massive doses are given may be present in large enough amounts to be active *This may explain certain of the contradictory* results reported in the treatment of nutritional macrocytic anemia with some of the more highly purified extracts

It is not proposed to go into the vast literature on the therapeutic use of liver and stomach extracts and of folic acid but only to deal with those aspects of this work which have a bearing on the etiology of the three conditions under consideration In the early days of crude extracts pernicious anemia nutritional macrocytic anemia and the macrocytic anemia of sprue all responded well to liver preparations It was originally thought that nutritional macrocytic anemia was due to a lack of Castle s extrinsic factor but doubt was thrown on this explanation when it was found that relatively purified extracts known to be potent in cases of pernicious anemia in relapse were completely inactive in the same or larger doses in the nutritional macrocytic anemia of monkeys, though campolon a very crude liver extract was active curatively in relatively small doses (Wills et al 1937) This work was confirmed by the same authors (1938) in a series of cases of nutritional macrocytic anemia in Bombay by Napier (1939) in Calcutta though he found that large doses of the same purified extract as that used by Wills and coworkers was active curatively in a few cases and by Giglioli in Central America (personal communication) Various workers in different parts of the world have shown that purified extracts may be active in certain types of *nutritional macro* cytic anemia as for example that occurring as a complication of pellagra (Moore et al 1944) and in enormous doses in the hemolytic type in women particularly in pregnant women in Macedonia but not in men in the same area (Fairley et al 1938 Foy 1939) Cases of nutritional macrocytic anemia also respond to marmite (autolysed yeast) and other so called good sources of the extrinsic factor when given by mouth Recently Castle and co workers (Watson and Castle 1946) have shown that more than one type of nutritional macrocytic anemia occurs one that responds as pernicious anemia does to the highly purified liver extracts given parenterally in normal doses another that responds to an unknown factor Wills factor as Castle calls it present in crude liver extracts and yeast given either orally or parenterally but not to purified liver extracts given parenterally and finally one that responds to purified liver extracts given parenterally when the dose is increased tenfold Such an enormous dose might contain sufficient Wills factor to produce a remission

In sprue the macrocytic anemia has been shown to respond to marmite by mouth to crude liver extracts by mouth and parenterally and also in many cases to purified liver extracts parenterally A high protein diet increases the hemopoietic effect

Finally folic acid has been shown to produce remarkable hemopoietic responses

in all three diseases In pernicious anemia folic acid either parenterally or orally induces in the vast majority of cases a maximal reticulocyte response followed by an immediate rise in the red and white cell counts The dose necessary to produce this effect is 5 to 10 mg daily or a single dose of 100 mg Jacobson (1947) by incubating folic acid with the enzyme xanthopterase has thereby enormously enhanced the hemopoietic activity of the folic acid and he suggests that by this means folic acid has either been converted into Castle liver principle itself or into another compound with great hemopoietic activity It is of interest in this connection to note that both the cases of pernicious anemia treated with the incubated material showed a steady rise in the red cell count and hemoglobin percentage to normal levels the count reaching the 5 0 million level and the hemoglobin a corresponding one This is in contrast to most workers experience with folic acid they *find that often after an excellent initial response it is impossible even* with increasing doses to get or maintain the blood at really optimal levels (Wilkinson 1947 Davidson and Girdwood 1947 Goldsmith 1947 Meyer 1947) Folic acid produces in all three of the anemias under consideration an immediate sense of well being of the same order as that produced by an active liver extract But again in the treatment of nutritional macrocytic anemia the blood fails to reach normal values and macrocytosis persists this is particularly so in cases of nutritional macrocytic anemia with diarrhea and also in cases of sprue though the general clinical improvement is remarkable (Davidson et al 1947 Morrison and Johnston 1947 Suarez et al 1947) It also has a miraculous effect in controlling the diarrhea of sprue and nutritional macrocytic anemia though analyses have shown that in spite of the steatorrhea being decreased there is no immediate alteration in fat absorption (Suarez Spies and Suarez 1947 Davidson et al 1947 personal cases)

In contrast to the general dramatic improvement is the complete ineffectiveness of folic acid treatment in arresting or preventing the development of symptoms of subacute combined degeneration (Spies and Stone 1947 Wilkinson 1947) The significance of these findings will be reviewed in the discussion on etiology of these three diseases

In brief it can be said that pernicious anemia *including the symptoms of subacute* combined degeneration can be successfully treated and health maintained with crude or purified liver extracts given parenterally or orally and proteolysed liver extract by mouth by different preparations of hog s stomach by mouth and by digests of beef muscle or autolysed yeast with normal human gastric juice Folic acid and its conjugated forms (Spies et al 1947) produces a remission which is often suboptimal of the hematologic symptoms an immediate sense of well being but no effect on the nervous symptoms the hematologic effect is said to be enhanced by incubation with xanthopterase

Nutritional macrocytic anemia both the nonhemolytic and hemolytic types seen in endemic form in many tropical countries responds to crude liver extracts parenterally or by mouth and to autolysed yeast extracts (Wills 1938 Napier 1939) Relapses do not take place after cessation of treatment if the diet is improved Folic acid has in the few cases reported the same action as in pernicious

anemia (das Gupta and Chatterjee 1946) * The hemolytic type seen in Macedonia is very resistant to treatment but some cases respond to enormous doses of both crude and purified liver extracts and to very large oral doses of marmite (Fairley et al , 1938 Foy and Kondi 1939)

The treatment of sprue has been studied by Fairley (1936) in cases largely from India and the Far East by a group of workers in Cuba and Puerto Rico (Spies et al 1946–47) and by the service authorities in India and the Far East where the disease was of relatively rapid onset (Leishman 1945 Maegraith et al 1945 Keele and Bound 1946) All agree that for op imal improvement a high protein diet with liver extract or folic acid are required In the critically ill, blood transfusion may be necessary and in the service cases sulphaguanidine often controlled the diarrhea The high protein diet leads to an improved nutrition as the absorption of protein does not appear to be affected

PATHOGENESIS AND DISCUSSION

Our present knowledge of the three clinical entities described supports in the author s opinion the view that they are three distinct separate diseases with essentially *different natural histories and pathological pictures It is now proposed* to discuss the evidence for this belief in the light of the facts set out in the previous paragraphs Authorities will not be quoted when they have already been given It is proposed to limit this discussion to the classic conditions as generally understood Pernicious anemia is a well recognized disease in which a persistent achylia gastrica is a diagnostic feature The title nutritional macrocytic anemia is limited to the disease as seen in endemic areas but will include sporadic cases occurring in other parts of the world but to simplify the discussion the sporadic cases of pernicious or macrocytic anemia of pregnancy will not be considered as it is felt that this group probably includes several different entities Tropical sprue is less well defined and recent experience in the services has led to the inclusion under this title of certain relatively acute conditions closely resembling sprue but not yet sufficiently worked out to be definitely included under the title of tropical sprue which for the purposes of this discussion will be limited to the classic disease as described by workers from Hillary in 1759 to Fairley in 1938 The service cases and sprue as seen in Puerto Rico will be discussed in relation to the classic picture

A consideration of the geographic ethnic and social distribution of these three diseases leads to the conclusion that they are three separate entities Pernicious anemia is a familial disease of persons of European descent with certain very definite genetic characteristics the distribution of the disease corresponding to that of the racial groups *affected chiefly fair and Nordic peoples Individuals of all classes* are affected In marked contrast is the distribution of nutritional macrocytic anemia This disease occurs mainly in tropical and subtropical lands but the

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distribution is associated with poverty a low calory largely or entirely vegetarian diet with pregnancy and lactation and also with certain diseases such as syphilis and particularly chronic malaria which result in a hypertrophied reticulo-endothelial system It also occurred in vegetarian Indian troops under the stress of service conditions and in Indian prisoners of war An anemia considered the animal counterpart of the human disease can be produced in monkeys by feeding with a diet based on one in common use among sufferers from this disease Pregnancy and lactation are known to increase the maternal requirements and these conditions will convert a latent deficiency into an overt one The relation of chronic malaria to this anemia will be discussed later These findings strongly suggest a direct nutritional origin in other words that this anemia is an unconditioned deficiency state The fact that this anemic syndrome is often associated with other symptoms including diarrhea often watery but sometimes fatty referable to a vitamin B deficiency supports the view that the anemia is a deficiency state The geographic ethnic and social distribution of tropical sprue presents a much more difficult problem Classic sprue has generally a gradual onset and is associated with residence in a warm climate but affects Europeans rather than pure Indians or Negroes The nature of the illness which is generally afebrile throughout throws little light on the etiology but the result of treatment suggests a deficiency state Though this certainly exists in the fully developed syndrome there is little evidence for a nutritional origin for the altered intestinal absorption which conditions the deficiency A more detailed examination of the distribution of the condition the heavy incidence in certain areas in the large endemic zones and even in certain houses combined with the fact that very many sprue patients come from the ranks of the well fed suggests a possible infective agent as the primary cause but this is purely conjectural A consideration of the outbreaks of acute sprue in the service is of interest in this connection though further study is necessary before these can be definitely considered the same clinical entity as classic tropical sprue Sprue in service personnel showed the same concentration in certain districts and sometimes in certain camps explosive outbreaks which assumed epidemic proportions in certain areas also occurred Leishman (1945) reports from Chittagong that nine separate units were affected some with a 50 per cent attack rate and one R A F unit had 10 per cent of its personnel down with diarrhea three weeks after its arrival in India which diarrhea rapidly turned to the sprue syndrome These findings are very strongly suggestive of an infective origin for this type of sprue

A study of the pathologic and biochemical findings in these diseases supports this idea of their essential individuality The pertinent findings in pernicious anemia are the atrophic changes in the fundus and cardia of the stomach with its associated achylia gastrica the increased plasma bilirubin the presence of methemalbumen in the plasma the distribution of iron in the tissues and other evidence of a hemolytic factor in the anemia and the changes in the central nervous system characteristic of subacute combined degeneration of the cord The atrophy of the gastric mucosa would appear to be the basic defect it involves all the coats of the stomach wall and results in a complete histamine resistant achlorhydria and an associated achylia There is no evidence of preceding inflammatory processes and

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* Since the writing of this paper Kemp (Lancet 2 351 1947) has reported 3 cases of nutritional macrocytic anemia who showed remarkable improvement with folic acid but were not studied long enough to show whether the blood level would have reached completely normal values and whether the macrocytosis still present with red cell counts at the 4 million level would have disappeared

in pernicious anemia in relapse This experiment indicates one of the factors that may influence the formation of the liver principle and may explain the pathogenesis of those macrocytic anemias which respond to nicotinic acid (Cook 1944)

The hemolytic process seen in pernicious anemia is of great importance both in a consideration of the essential pathology of the disease and in considering its relationship to nutritional macrocytic anemia and sprue There is little to explain the increased serum bilirubin and other evidence of increased red cell destruction except that it ceases under appropriate treatment with liver hog s stomach or folic acid and might therefore be thought to be due to the nature of the macrocytes There is however no increased fragility of the red cells and no hemolysis has been demonstrated in the blood stream but there is evidence of active phagocytosis of the red cells in the reticulo-endothelial cells of the bone marrow liver and spleen which might account for some of the increased bilirubin in the circulation But the presence of methemalbuminemia and a positive Schumm test would suggest some additional intravascular hemolysis (Fairley 1941) With certain exceptions already mentioned there is no evidence of hemolysis in either nutritional macrocytic anemia or sprue and as the megaloblastic reaction is generally considered identical in all these anemias it is unlikely that the hemolysis in pernicious anemia is due entirely to the nature of the cell It would appear more likely that it is due to an association of an overactive reticulo-endothelial system with abnormal cells though the cause of this hypothetical increased activity is unknown The evidence of increased hemolysis seen in cases of nutritional macrocytic anemia occurring in areas of endemic malaria supports this view

Tropical sprue is a dramatic disease in which spectacular pathologic changes in the tissues might be expected but postmortem examinations have failed to reveal any anatomic changes except those in the bone marrow and those of extreme inanition the loss of fat and shrunken organs There are no lesions of the nervous system as in pernicious anemia Histologic examination of material from the gastrointestinal tract fixed immediately after death has shown essentially normal structures The changes in the gut are functional and not anatomic In a brilliant review of recent work Stannu (1942) has marshalled the evidence for a failure of phosphorylation of fatty acids glycerol and glucose being the basic lesion the point of functional breakdown There is much evidence in support of this view Macgraith and colleagues (1945) have shown in cases of sprue like conditions that in the active phase the absorption of glucose is grossly impaired though that of fructose is not suggesting that there is an impairment of phosphorylation of glucose although the diffusion of sugars across the membrane is unaffected Leishman (1945) suggests that the vitamins riboflavin and nicotinic acid are concerned in this process of phosphorylation in their phosphorylated forms as co-enzyme I and yellow oxidase they take part in cellular metabolism acting as H acceptors or rejectors and Leishman thinks it is possible that they may catalyse the process of phosphorylation It is possible that some other member of the vitamin B_2 complex takes part in this process But if the failure in phosphorylation is due to deficiency of any B vitamin how does this deficiency arise in well fed people and why did it not occur among prisoners of war in Japanese hands who suffered so badly from

Magnus (1938) thinks the evidence points to the change being the final stage of an atrophic process the cause of which is unknown but might be the end result of some endocrine or nutritional deficiency or might even be congenital in origin The evidence for the genetic factor is the familial and racial incidence and the lack of any indications of an infective nutritional or endocrine origin Idiopathic hypochromic anemia an iron deficiency anemia associated with achylia gastrica also occurs in families subject to pernicious anemia (Wintrobe and Beebe 1933) In sprue an atrophy of the gastric mucosa has been reported by the Puerto Rico workers but the evidence points to it being secondary to the disease as it occurs only in the fully developed syndrome Mackie and Fairley from a study of specially fixed material report a normal mucosa In the nutritional macrocytic anemia of monkeys there is no significant change in the gastric mucosa and presumably the mucosa is normal in the corresponding human conditions Test meal findings confirm the essential normality of the gastric mucosa in most cases of sprue and nutritional macrocytic anemia The defect in the gastric mucosa seen in pernicious anemia according to Castle s well known theory produces a lack of his intrinsic factor a substance with enzymic properties and it is this deficiency that leads to the failure of the formation of the liver principle Castle s work has shown the mode of action of this intrinsic factor but neither it nor the extrinsic factor have been isolated any more than the liver principle itself

This defect in the gastric secretion with its interference with the formation of the liver principle and possibly with another principle essential for the proper functioning of the nervous system appears to be the basic lesion in pernicious anemia but unless we accept the view that a certain variable time is necessary for the postulated genetic factor to bring about this gastric atrophy it is necessary to look further for a factor producing this atrophy from the evidence it seems that there is little to suggest an inflammatory one This same time factor in the development of the symptoms of pernicious anemia appears to operate in those cases which develop the disease after total gastrectomy a period as long as ten to fifteen years occurring between the time of operation and the time of development of symptoms (Meyer et al 1941) This time lag is also unexplained In this connection Rhoads s (1933) experiments on the production of a syndrome resembling pernicious anemia in hogs by feeding modified black tongue diets are of interest The deficiency not only produced tongue changes a macrocytic anemia and nerve lesions but a histamine resistant achlorhydria this syndrome though it resembled that of pernicious anemia differs from it in that the gastric changes are reversible and the condition could be cured whereas in true pernicious anemia replacement therapy is always necessary the primary lesion being irreversible

Another experiment of interest in this connection is that of Petri (1944) and co workers these authors have shown in experiments on dogs and swine that total gastrectomy produces signs of pellagra due apparently to interference with absorption of nicotinic acid Furthermore the livers from such pigs were ineffective in the treatment of pernicious anemia However nicotinic acid by the parenteral route compensated in these animals for the absence of gastric secretion the livers from gastrectomized pigs receiving parenteral nicotinic acid being fully effective

Other factors may also be concerned and a deficiency in any one of these might produce a similar failure in the function of the liver principle

Finally it is interesting to consider these three entities from the point of view of preventive medicine If a genetic defect is the ultimate cause of pernicious anemia then only selective breeding at present a Utopian and risky measure can eradicate it If as seems highly probable nutritional macrocytic anemia is a deficiency disease due to a lack in the diet of some factor associated with good biologic protein then improved economic conditions with the improved diet that always goes with them should eradicate the disease except in those cases where *religion* limits the diet when only a new revelation can assist But the problem of sprue awaits further work as until we know the nature of the original cause of the intestinal breakdown it is impossible to take preventive measures

SUMMARY

The following tentative conclusions as to the relationship of pernicious anemia nutritional macrocytic anemia and tropical sprue to one another and their patho genesis are drawn from a study of the literature and from unpublished work

1 That these three clinical conditions are three distinct entities possessing a common characteristic in the presence of a panhemopoietic dystrophy characterized by a megaloblastic erythropoiesis and corresponding changes in the myeloid cells and platelets

2 That this panhemopoietic dystrophy possibly results from the breakdown of an intracellular enzyme system but that the deficiencies causing the breakdown differ in pernicious anemia the liver principle is apparently at fault in endemic nutritional macrocytic anemia another unidentified factor is missing in sprue either or both may be at fault

3 Folic acid is active therapeutically in all three diseases but in all it generally fails to restore completely normal blood levels

4 Pernicious anemia is probably due to a genetic defect which produces an atrophy of the gastric mucosa As a consequence of this interference with gastric function there is a failure in the formation or absorption of the liver factor and possibly of another neurotrophic factor which failure results in the development *of a macrocytic megaloblastic anemia and the characteristic changes in the nervous* system Indefinite replacement therapy is necessary as the changes in the gastric mucosa are irreversible The cause of the increased hemolysis is unknown

5 Endemic nutritional macrocytic anemia is an unconditioned food deficiency the deficiency being in a factor other than the liver principle possibly a co-enzyme present in or associated with good biologic protein and the vitamin B complex There are no characteristic pathologic changes except those of the hemopoietic organs which are not specific to the disease A hemolytic type of the disease occurs in areas of high malarial incidence After successful treatment the disease does not relapse if the diet is satisfactory Pregnant women are particularly liable to develop the disease

6 Tropical sprue is due to a functional disorder of the intestine possibly pri marily a failure in phosphorylation of fatty acids glycerol and glucose Diarrhea

deficiencies of these vitamins Leishman points out in this connection that the B vitamins are synthesized in very appreciable amounts in the gut and that a change of diet or dysentery might alter the balance of the intestinal flora and hence of vitamin synthesis Work along these lines might be illuminating

Pathologic and biochemical observations throw little light on the essential pathology in nutritional macrocytic anemia As in pernicious anemia and sprue the hemopoietic organs show the changes characteristic of a macrocytic anemia but both in nutritional macrocytic anemia and in sprue the bone marrow from the tibia (the bone examined) may show gelatinous changes as well as the extension of the red marrow characteristic of pernicious anemia Beyond these changes and those due to inanition there are no obvious pathologic lesions Magnus has shown the presence of a normal gastric mucosa in material from monkeys suffering from nutritional macrocytic anemia In man the gastric acidity and the blood sugar levels after oral glucose fall within normal limits There is no evidence of increased hemolysis except in areas with a high incidence of malaria Fairley and Foy have given detailed accounts of this type in which the increased serum bilirubin values a positive Schumm test the increased urobilin output the yellow color of skin and body fat are all indications of an increased hemolysis The spleen is enormously increased in size frequently the liver also and postmortem material has shown a hyperactive reticulo endothelial system Fairley sums up the picture thus A reticulo endothelial system irritated activated and hypertrophied as a result of repeated malarial infections phagocytoses these non parasitized abnormal corpuscles in considerable numbers producing a haemolytic anaemia The corpuscles are abnormal macrocytes the result apparently of a marrow rendered megaloblastic by a deficiency of the same nature as that operation in uncomplicated nutritional macrocytic anemia

It has been suggested in the preceding paragraphs that these three diseases all exhibiting an apparently identical panhemopoietic dystrophy are distinct clinical entities It can be postulated that this dyshemopoietic anemia probably results from the breakdown of some intracellular enzyme system in which the liver principle plays an important part The relation of folic acid to this system still awaits solution but the fact that it has such remarkable if limited hemopoietic activity in all three conditions as well as a dramatic effect on the well being of the patient and on diarrhea if present shows that it plays an all important role in rectifying the faulty cellular metabolism The liver principle is active curatively in pernicious anemia but does not seem to be the missing factor in nutritional macrocytic anemia as it has been shown to be inactive in this anemia in man and monkeys in doses equivalent to or greater than those giving maximal responses in cases of pernicious anemia with similar initial blood levels That this factor Wills factor as Castle calls it is not Castle s extrinsic factor follows from the fact that the liver principle in purified preparations is inactive in nutritional macrocytic anemia Recent work on the relationship of folic acid to vitamin M deficiency in monkeys (Day et al 1945 and 1946 Wilson et al 1946) suggests that possibly folic acid is the missing factor It is possible that Wills factor is an activator or co enzyme in an enzyme system in which the liver principle plays the important part

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with characteristic stools and a macrocytic anemia are characteristic findings The macrocytic anemia may be due to a failure in absorption of one or more essential hemopoietic factors or to a lack of Castle s intrinsic factor The cause of the functional breakdown is unknown Treatment is with a high protein diet and liver extracts Relapses are common

My thanks are due to my colleagues for carrying on my work while I wrote this paper

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families many cases occur of both cryptogenetic anemia and pernicious anemia due to worm The cause of this has been considered a special constitutional disposition for this type of anemia It may be that this disposition consists in a deficient production of intrinsic factor

2 The Extrinsic Factor

a) *Clinical observations* Experience in Finland shows that worm carriers can have pernicious anemia even though their food contains a sufficient amount of protein of different kinds Apparently lack of extrinsic factor is not essential for the occurrence of pernicious tapeworm anemia (in contrast to some other conditions such as for example the nutritional tropical pernicious anemia) However certain facts do support the idea that a relative deficiency in extrinsic factor can contribute to the rise of the disease G Totterman found in his material a higher frequency of pernicious tapeworm anemia in 1942 than in 1943 In the former year the food situation in our country was particularly serious there was a special lack of proteins The next year the situation had improved considerably My own experience agrees with that of Tötterman s In some cases of worm anemia I have seen a slight reticulocytosis and even a certain improvement of the erythrocyte count during the administration of meat This was the case with soldiers who came for treatment directly from the front Cramer (1922) observed pernicious tapeworm anemia at the same time in 3 sisters who by reason of some mental peculiarity lived like hermits and fed themselves with an extremely insufficient diet

b) *The course of remission after removal of the worm in the absence of extrinsic factor* It is logical to assume that there will be no remission after the worm is expelled if the patient shortly before and after the worm cure has taken food containing no extrinsic factor The correctness of this reasoning has been shown in a series of twelve tests When the patients were admitted to the hospital they were placed on a basic diet as free from extrinsic factor as possible After some days they were given a worm cure Very insignificant signs of blood regeneration or none at all were observed even after twelve to fifteen days On the contrary the blood picture often became progressively more abnormal As soon as substances known or believed to contain extrinsic factor were added to the diet a marked reticulocytosis began and the blood picture improved rapidly It has been proved that this is true when meat milk Hammarsten s casein pepton brewer s yeast and concentrated yeast extract and to a lesser degree soy bean protein were added to the diet These observations confirm Castle s theory that both intrinsic and extrinsic factor are necessary

The method furnishes a means of testing substances for their content of extrinsic factor

One practical conclusion is that after worm cure patients with worm anemia must be given a diet rich in proteins if a rapid remission is to be expected

3 The Interaction between the Intrinsic and Extrinsic Factors

Castle s test has not been previously carried out on patients with pernicious tapeworm anemia yet this experiment is of great importance It is conceivable that

worm cure the megaloblastosis in the bone marrow *gives way to a normoblastic* type of regeneration

The most rational therapy in pernicious tapeworm anemia is the simple worm cure Only in very severe anemia is it necessary to start treatment with liver in jections and not expel the worm until after the blood picture has improved In such cases however it is difficult to decide afterwards whether the pernicious anemia was caused by the worm or not Only if the patient is very young and/or has free hydrochloric acid in the gastric contents can one be to some extent certain that the pernicious anemia was caused by the tapeworm

The fact that the pernicious tapeworm anemia is completely cured after the worm is expelled can be explained in no other way than that the patients have access to all the substances required for the endogenous formation of the antianemic prin ciple Evidently these substances are available directly after elimination of the worm as indicated by the promptness with which the remission begins thereafter If the food contains the extrinsic factor this must imply above all that the intrinsic factor becomes at once available

b) *Castle s test with gastric juice from patients with pernicious tapeworm anemia* Hernberg (1936–1941) has shown that the gastric juice in patients with pernicious tapeworm anemia as well as in persons who have had it contains intrinsic factor Mixed with meat such gastric juices produce a typical remission when given to patients with cryptogenetic pernicious anemia

c) *In vitro experiments* The author has made some investigations of the proteo lytic gastric enzyme active at neutral reaction according to the method given by Taylor et al (1938) and has found (1940) that this enzyme occurs in pernicious tapeworm anemia as well as in cryptogenetic pernicious anemia though in the latter cases the total amount of the gastric secretion is very much reduced It has been suggested that this enzyme is identical with the intrinsic factor My results have been confirmed by Helander (1945) Hernberg (1939) with Lasch s reaction has obtained similar results in the gastric juice from patients with pernicious tape worm anemia

The investigations here reported all support the idea that the gastric juice of patients with pernicious tapeworm anemia contains intrinsic factor In spite of this and in spite of the fact that the amount of gastric juice is often normal an anemia has arisen Apparently the pernicious anemia in tapeworm carriers is not *caused by cessation of the secretion of the intrinsic factor because of the presence of* the worm However it is evident that some inhibition of the gastric juice secre tion may occur in connection with pernicious tapeworm anemia for in some cases free hydrochloric acid reappears in the gastric contents after the remission in pa tients who showed achlorhydria while the anemia was apparent

The author has been unable to find any difference in the speed of remission after a worm cure in patients with achlorhydria and those with normal gastric secretion

It seems possible that a decreased secretion of intrinsic factor may facilitate the occurrence of a pernicious anemia in connection with tapeworm infestation It is well known that some people who have had a pernicious tapeworm anemia when young have later fallen ill with a cryptogenetic form of th disea In some

the worm toxins absorbed from the intestine may destroy the entire quantity of antianemic liver factor available in the body but this is an improbable theory

It is a priori somewhat improbable that the tapeworm can injure the liver factor partly because it is rather stable and partly because we know that the administration of liver preparations both parenterally and per os quickly cures a pernicious tapeworm anemia

I have incubated ordinary injectable liver extracts together with worm in vitro at 37 C for some days and could not at least in this way prove any decrease of their antianemic effect

It appears then that the lack of the liver factor is not the result of destruction by the tapeworm itself nor by toxins from the worm

5 Folic Acid

I have treated 4 cases of pernicious tapeworm anemia with folic acid per os An excellent remission was obtained in all cases with doses of 20–30 mg daily for 7–10 days showing that folic acid also is not injured by the worm

6 The Absorption

The clinical picture in pernicious tapeworm anemia gives no reason to believe that the absorption in this disease is impaired Carriers of Diphyllobothrium latum seldom suffer from severe intestinal disturbances Worm carriers with and without anemia do not differ from each other in this respect In no case are the conditions comparable with those in sprue intestinal anastomoses etc

The glucose tolerance test has been carried out in 4 cases of pernicious tapeworm anemia both before the worm cure and after the blood had become normal In all cases the blood sugar curve had a normal course both before and after the worm cure thus it was not possible to show that there was any disturbance in the glucose absorption

7 Experimental Feeding with Tapeworm Preparation

The effect on the blood of giving worm preparations per os or parenterally has of course been studied in both animals and humans T W Tallqvist (1907) experimented on himself in this way and G Totterman (1938–1940) has published a large series of tests Both have thought they saw a certain anemising effect from the preparations they used I am not convinced of the correctness of their conclusions for reasons stated in another publication

The problem has been attacked by attempting to answer the following questions (1) Is the antianemic effect of the mixture of gastric juice and meat nullified if worm is added? (2) Is the remission after the worm cure absent in worm anemia patients if the worm preparation is given per os?

The mixtures of gastric juice and meat (or yeast extract) were prepared in the same way as described earlier The subjects were patients with untreated cryptogenetic pernicious anemia First they were treated for eight days with gastric juice plus meat (or yeast extract) with the addition of a considerable amount of fresh or dried Diphyllobothrium latum The remission was always splendid During the

the worm in the intestinal canal prevents the interaction between extrinsic and intrinsic factors and in this way gives rise to the pernicious anemia If this is true it would be expected th_t no remission would occur when a patient is given a mixture of meat and gastric juice This would indicate that the worm has been able to destroy the effect of these substances supplied from outside in the same way as it prevents the body s own intrinsic factor from interacting with extrinsic factor in the patient s ordinary food If a fresh mixture of gastric juice and meat proves to be ineffective while the same mixture incub_ted for six hours at 37° C does have an anti_nemic effect the conclusion might be drawn that by means of the enzyme activity in vitro some new substance is formed which the worm is unable to injure

A series of 14 tests was carried out to clear up this question Me_t (150 Gm per day) or in some tests yeast extract was used as the source of the extrinsic factor The daily amount of gastric juice with which the meat or yeast was mixed w_s 150-175 ml Each test period lasted eight days During the first test period a nonincubated mixture was given

In some cases of cryptogenetic pernicious anemia these tests produced a splendid remission The effect was equally good wh-ther the mixture was incubated previously or not On the other hand the test results were clearly negative in cases of p rnicious tapeworm _nemia Neither fresh nor incubated mixtures of meat and gastric juice produced any remission In some cases the identical gastric juice was used as in parallel tests with cryptogenetic pernicious anemia The remission occurred only after the worm had been expelled

In one case of pernicious tapeworm anemia 100 ml of gastric juice was brought up daily after insulin stimulation and was incubated with 150 Gm of meat for six hours after which the mixture was administered to the patient Not even in this way could any remission be produced

These observations give strong support to the ide_ that the worm in the intestinal canal is capable of preventing interaction between the extrinsic and intrinsic factors and that such an inhibition can be deemed to be the reason for the pernicious anemia

The fact that incubation does not involve an improvement of the antianemia effect of meat and g stric juice confirms the assumption that the antianemic principle cannot be formed in vitro but only in vivo It is possible that the interaction b tween extrinsic and intrinsic factors takes place in the intestinal wall (Formijnex 1940) Perh ps this interaction is not a simple enzyme re_ction

4 The Liver Factor

If the worm in pernicious tapeworm _nemia is expelled and the formation of new antianemic factor is prevented by giving a diet free from extrinsic factor then as already stated there is no blood remission This shows that the liver must be deprived of its stock of antianemic factor for if any were present blood regeneration should take place after the anemia producing worm had been removed independently of the supply of intrinsic and extrinsic f_ctors

It is conceivable that the worm may destroy the _ntianemic factor at the place where it is assumed to be formed i e in the intestine Another possibility is th_t

9 The Localization of the Tapeworm in the Intestinal Canal

Presumably the worm cannot inhibit the reaction between extrinsic and intrinsic factor unless the worm is present at the place where the interaction occurs Now the question arises Where in the intestinal canal is the worm to be found? Very uncertain information on this point is available Experience from operations and autopsy in general indicate that the worm has been chiefly observed in the ileum but there are no systematic observations of this fact Sometimes it happens that Diphyllobothrium latum is vomited which shows that at least occasionally it can be very high up in the intestine To investigate this question the author made a series of intestinal intubations As the Diphyllobothrium latum produces large quantities of eggs it was relatively easy to determine at what distance from the mouth the first eggs could be aspirated In many cases small pieces of the worm itself were aspirated at the same time Of course it is not possible to calculate in this way the highest point in the intestine where the worm is attached Although no eggs are produced from the highest segments of the worm yet I have been convinced that results can be obtained which allow comparison between different cases

Table 1 *Distance from Mouth (cm) where Ova and/or Proglottids of Diphyllobothrium Latum Were Found*

Group 1	Group 2	Group 3	Group 4
No anemia	Nonpernicious anemia	Pernicious tapeworm anemia in relapse	Pernicious tapeworm anemia in spontaneous remission
235	334	135	32
235	180	1 0	242
230	142	1 0	95
190		120	
180		115	
[illegible]		115	
165		110	
150		105	
145		105	
3		95	

The intubations were carried out on 16 worm carriers who were divided into 4 groups as appears in table 1 in which the results are also summarized

The results show that in manifest pernicious anemia the worm is found higher up in the intestine than otherwise Perhaps one can imagine that in that region it is better able to interfere with the interaction between extrinsic and intrinsic factors How high up in the intestine the worm must be for it to inhibit this reaction is difficult to determine My results favor the opinion that a critical limit lies 140–150 cm from the mouth which ought to be about the borderline between the jejunum and the ileum

10 An Attempt to Explain the Pathogenesis of the Pernicious Tapeworm Anemia

As described above it appears to be possible that the Diphyllobothrium latum causes pernicious anemia by inhibiting the interaction between extrinsic and intrin

*following period with gastric juice plus meat (or yeast extract) without the addi*tion of worm no new reticulocytosis was observed and the blood regeneration was no more rapid than during the first test period

In one case of worm anemia the patient, after the worm cure was given worm powder per os in increasing amounts In spite of this the blood improved in the usual way

In some tests the worm anemia patients were kept on a diet free from extrinsic factor, were given worm cure and then for eight days extrinsic factor in the form of yeast extract with the addition of worm powder In spite of this addition the blood improved rapidly

In connection with these tests worm powder was mixed with hog s stomach in order to investigate the possibility of loss of antianemic effect In spite of the addition of powdered worms in two such tests the hog s stomach still had a marked antianemic effect

It was thus shown that addition of worm is unable to destroy the antianemic effect of mixtures of gastric juice and extrinsic factor or of stomach preparations Moreover the presence of worm does not prevent the remission after the elimination of the worm in worm anemia This fact has been interpreted to mean that the inhibition of the interaction between the intrinsic and extrinsic factors can be produced only by the living worm in its natural surroundings at the place where the interaction occurs

8 Inhibition in Vitro of the Proteolytic Activity of Gastric Juice at Neutral Reaction

The gastric protease which is active at a pH range from 5 to 9 is greatly inhibited in its hydrolytic capacity in vitro after the addition of even relatively small amounts of Diphyllobothrium latum The inhibitory substance is destroyed by heating to 80° C for twenty minutes It is not dialyzable and is not soluble in ether nor in 98 per cent ethyl alcohol It cannot be precipitated with 50 per cent alcohol but can be precipitated quantitatively in 90 per cent alcohol

*The gastric protease in question has been assumed to be identical with the in*trinsic factor and the hydrolysis of casein in vitro has been considered as corresponding to the interaction between the intrinsic and extrinsic factors in vivo It has not yet been possible to prove this assumption As stated above it seems probable that such interaction cannot occur in vitro but only in the intestinal canal

There is thus a descrepancy as follows (a) The living worm in situ seems to inhibit the interaction between the extrinsic and intrinsic factors (b) the administration *per os* of worm preparations does not inhibit this interaction while again (c) the addition of worm in vitro inhibits the proteolytic activity of gastric juice at neutral reaction It seems that this discrepancy cannot be explained until we have more detailed knowledge of the different substances here concerned The exact chemical nature of the extrinsic factor intrinsic factor and the tapeworm toxin are as yet unknown

9 THE LOCALIZATION OF THE TAPEWORM IN THE INTESTINAL CANAL

Presumably the worm cannot inhibit the reaction between extrinsic and intrinsic factor unless the worm is present at the place where the interaction occurs Now the question arises Where in the intestinal canal is the worm to be found? Very uncertain information on this point is available Experience from operations and autopsy in general indicate that the worm has been chiefly observed in the ileum but there are no systematic observations of this fact Sometimes it happens that Diphyllobothrium latum is vomited which shows that at least occasionally it can be very high up in the intestine To investigate this question the author made a series of intestinal intubations As the Diphyllobothrium latum produces large quantities of eggs it was relatively easy to determine at what distance from the

TABLE 1 —*Dista ce from Mo th (cm) wh e Ova and/ r Pr gl ttids of Diphyllob thrium Latum W e Fou d*

(p 1 \ a m	C p \ pe ci em a	(p 3 P t pe rm m m f t	(p 4 Pern iou tapew rm a m in po ta eo s t m
235	334	135	32)
235	180	120	240
230	140	120	105
180		120	
180		115	
180		115	
165		110	
150		105	
145		105	
130		95	

mouth the first eggs could be aspirated In many cases small p eces of the worm it self were aspirated at the same time Of course it is not possible to calcul te in this way the highest point in the intestine where the worm is attached Although no eggs are produced from the highest segments of the worm yet I have been con vinced that results can be obtained which allow comparison between different cases

The intubations were carried out on 26 worm carriers who were divided into 4 groups as appears in table 1 in which the results are also summarized

The results show that in manifest pernicious anemia the worm is found higher up in the intestine than otherwise Perhaps one can imagine that in th t region it is better able to interfere with the interaction between extrinsic and intrinsic factors How high up in the intestine the worm must be for it to inhibit this reaction is diffi cult to determine My results favor the opinion that a critic l limit lies 140–150 cm from the mouth which ought to be bout the borderline between the jejunum and the ileum

10 AN ATTEMPT TO EXPLAIN THE PATHOGENESIS OF THE PERNICIOUS TAPEWORM ANEMIA

As described above it appears to be possible that the Diphyllobothrium latum causes pernicious anemia by inhibiting the interaction between extrinsic and intrin

sic factors, but that this reaction can occur only if the worm is sufficiently high in the intestine However as stated previously there is reason to presume that the amount of extrinsic factor in the food and of intrinsic factor in the gastrointestinal canal is also of some importance Thus whether anemia occurs or not would depend on a definite correlation among these three determinants the amount of extrinsic factor the amount of intrinsic factor and the worm s high or low position in the intestine

Finally a time factor must also be taken into consideration The formation of antianemic factor must have been inhibited for such a long time that the liver is wholly deprived of it Only then is there reason to expect that the anemia will manifest itself

It must be emphasized that in the great majority of cases of pernicious tapeworm anemia there is no basis for the assumption that either a defective diet or a hereditary disposition are to be reckoned with as co operating causes Most tapeworm anemia patients do *not* fall ill later with a cryptogenetic anemia I can therefore not confirm the correctness of Birkeland s conclusion that it seems appropriate to classify surviving patients with Diphyllobothrium anemia as suffering from abortive forms of genuine pernicious anemia My view of the problem is that in principle any worm carrier whosoever can get a pernicious tapeworm anemia if only the worm—ceteris paribus—is high enough up in the intestine If the worm is expelled a complete restitution can follow

The theory I have formulated explains—in my opinion—the following circumstances which have been specially put forward by Saltzman (1924) and which have hitherto been difficult to interpret

a) *A person can carry Diphyllobothrium latum for many years before he falls ill with pernicious anemia* The explanation of this can be that the worm for one reason or another has invaded the upper parts of the intestine sometimes possibly as a reinfestation

b) *A person who has had pernicious worm anemia and becomes well after the worm is expelled does not necessarily get anemia if he is again infested with worm* At the reinfestation it may happen that the worm is only in the lowest parts of the intestine

c) *A worm expelled from an anemia patient is often disintegrating and discolored* Sometimes no worm at all is seen in the feces According to my theory this disintegration can be due to the fact that the worm being higher up in the intestine has had a longer distance to go before it was expelled During its passage through the intestine it has had to undergo a strong autolytic decomposition and is also affected more by the digestive enzymes than if it had been in the lower part of the small intestine and only had to pass through the colon where the enzymes are less active

d) *The amount of worm is not in correlation with the occurrence of anemia* A small amount of worm can cause anemia if it is sufficiently high up in the intestine while a large amount does not necessarily do so if it is collected in the lower part of the small intestine Yet cases with very large amounts of worm 80 100 M and more are often accompanied by anemia In such cases it can be imagined that the worm because of its great volume, has been forced upwards towards the jejunum

e) *Spontaneous remission* with a return to normal blood values are not rare in worm

anemia The explanation of this can be that the worm had deserted the upper parts of the small intestine and wandered down towards the ileum In my 3 cases of this type the worm was found just as low in the intestine as in nonanemic worm carriers (cf table 1)

f) *Remission after an incomplete worm cure can also occur* A filicin cure can fail in such a way that only a small amount of the worm or none at all is expelled and after the cure worm eggs can still be seen in the feces In spite of this the blood improves At a later worm cure—after the blood picture has become normal—a considerable amount of worm is often removed In such cases—according to my idea—the worm at the first unsuccessful cure was driven from the upper part of the intestine but remained in the lower part where it was no longer able to exercise its anemia producing effect

This idea was confirmed by the following test In one case of manifest pernicious worm anemia the eggs were found 115 cm from the mouth Through the intestinal tube 2 Gm filicin emulsion were instilled The worm was not expelled and the feces continued to contain worm egg but they could not be demonstrated as present at the former depth (115 cm) A few days after the filicin cure a marked reticulocytosis began and the blood picture improved rapidly The tube was then allowed to glide farther in and worm eggs were not found till 200 cm from the mouth that is far down in the ileum Following another treatment with filicin 31 M of ordinary looking Diphyllobothrium latum were expelled

Comment

According to one earlier theory the occurrence of worm anemia may be due to a change in the character of the parasite possibly an abnormal disintegration of the worm in the intestine but I have been unable to find any signs of such a disintegration The worm segments which I sometimes aspirated at intubation from worm anemia patients have been very motile and of ordinary appearance According to other theories the cause of the anemia lay in the host These theories have presumed a varying permeability of the intestinal wall to the worm toxin a special individual susceptibility of the hemopoietic organs to it or an allergic preparedness G Totterman has classed the pernicious tapeworm anemia with the malignant granulocytopenia due to the use of amidopyrine Apart from the fact that I find the experimental basis of these theories defective none of them seem to me to explain the worm anemia problem satisfactorily It appears artificial to conceive of the macrocytic anemia with its megaloblastosis as an allergic reaction Another fact that tells against the toxic and allergo toxic theories is that the anemia can be cured with liver or stomach preparations without expulsion of the worm Again on the basis of them it is difficult to explain why no remission occurs after the worm cure unless extrinsic factor is available The circumstances listed under Section 10 (a–f) above are also not easy to explain

There is no doubt that Diphyllobothrium latum contains a powerful poison If one handles fresh worm with unprotected hands the skin is greatly irritated If one places a small amount of dried and pulverized worm on the tongue there is a feeling of burning The inhalation of worm powder has been proved to produce nausea

fever rhinitis asthmatic cough and eosinophilia in the blood A worm carrier often suffers from giddiness various nervous manifestations and nausea, has eosinophilia (which may or may not be absent in anemia cases) and shows serological changes Like the macrocytic nonpernicious anemia described by G Totterman these phenomena can be considered as the expression of the effects of toxic activity resembling those which condition the rise of pernicious anemia Thus according to my idea the inhibition of the interaction between extrinsic and intrinsic factors is only *one* expression of the worm s toxicity

Tests on animals with worm preparation injections also bear witness to the toxicity of the worm Among other things it appears to contain a hemolytic toxin Yet it has not been possible with these tests to produce an anemia which directly corresponds to the Addisonian anemia in man

Much confusion in the discussions could I believe be avoided if the ability of the worm to produce pernicious anemia was consistently kept separate from its other toxic properties

Certain details in the tapeworm anemia problem still await solution Like the cryptogenetic pernicious anemia the pernicious tapeworm anemia shows definite variations in its seasonal distribution It is most usual during the period from March to August Illustrative curves with which my own experience agrees are to be found in Birkeland s monograph It is at present impossible to decide to what extent these seasonal variations depend on circumstances in the patient himself on light conditions on the contents in the diet of protein folic acid (as suggested by Waldenstrom) or other substances A racial factor (in connection with pigment metabolism) must also be taken into consideration in both forms of pernicious anemia

The chemical nature of the worm s toxin is still unknown If that could be determined it might be possible to get a better idea of the process by which the poison interferes with the interaction between the extrinsic and the intrinsic factors

I have tried to show here that the investigation of pernicious worm anemia has not only a local interest but can also contribute to the elucidation of the whole great question of the macrocytic and megaloblastic anemias which respond to liver treatment and which are termed pernicious

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REFRACTORY MEGALOBLASTIC ANEMIA

By L S P Davidson, B A M D F R C P Ed and Lond F R S Ed

THE TERM refractory anemia was introduced by Bomford and Rhoads (1941) for anemias of a wide variety of types that were refractory either temporarily or permanently to hematinic therapy In 1943 Davidson Davis and Innes published a series of papers entitled Studies in Refractory Anaemia which dealt with the problem of classification on the basis of examination of the bone marrow by sternal puncture and divided the anemias refractory to liver extracts into two main groups namely (1) refractory anemias with hypocellular normoblastic marrows and (2) refractory anemias with hypercullular megaloblastic marrows Of particular significance was their finding that the prognosis was vastly different in the two groups Thus of 16 patients in Group 1 11 died of progressive anemia within a few months while of 16 cases in Group 2 all eventually made a complete recovery Intensive treatment with large amounts of liver extract supplemented with iron and vitamins and repeated blood transfusions was required for long periods if such satisfactory results were to be obtained The long period of illness during which life was continuously in danger indicated the need for some therapeutic agent which would cause a prompt remission comparable to that obtained with parenteral liver therapy in the relapse stage of Addisonian pernicious anemia

In this paper the term refractory megaloblastic anemia is confined to cases of megaloblastic anemia which failed to respond hematologically and clinically to the parenteral administration of an amount of liver extract which has been proved to produce an optimal response in cases of Addisonian pernicious anemia The test preparation employed was Anahaemin marketed by British Drug Houses Ltd which has been found by the writer to be potent in a dose of 2 cc when administered parenterally in a large number of cases studied during the past ten years Every patient with refractory megaloblastic anemia received at least twice this dose after admission to hospital In addition many cases had received large amounts of potent liver extracts prior to being referred to us for investigation of their failure to respond Since infections intoxications and advanced arteriosclerosis are known to inhibit or delay the response to parenteral liver therapy patients exhibiting any of these complications *were not included in the group of refractory megaloblastic* anemia discussed below

For many years the writer has suggested that chemical purification of liver extracts for parenteral use removes some essential factor which is necessary for the restoration of normal blood formation in certain cases of megaloblastic anemia which have failed to respond to potent liver extract given parenterally The following case history of a patient seen by the writer nearly fifteen years ago illustrates this problem very clearly

The patient was a middle aged business man who had worked in India for many years and had always been in good health until one year before the present illnes His case notes from Calcutta indicated that

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a year previously he had had an atta k of dysentery from which he apparently recovered complet ly A f w months later h began to feel tired and breathl ss on exertion His tongue becam sore and his bowels loose The report from a medi al specialist in Calcutta indicated that he had a moderate degree of macro cyti anemia and free hydrochloric acid was present in the gastric juice Desp t all treatment he con tinued to lose weight and strength rapidly and was sent home to Scotland for investigation of th cause of his illness Wh n I first saw the patient he was extremely emaciated having lost 6 stone in weight during the previous twelve months H was passing pale greasy bulky stools and the blood picture was typical of Addisonian pernicious anemia His red cells numbered 1 M and his Hb 25 per cent A hista min fast achl rhydria was present He was diagnosed as suffering from tropical sprue and given a low fat diet supplemented by vitamins and iron Parenteral treatment with the liver extract Campolon was started This resulted in a rise of reticulocytes to 15 per cent but no subsequent increase in red cells or Hb occurred The patient was desperately ill and life had to be maintained by blood transfusions Parenteral liver therapy was continued but was totally ineffective The patient s diet was then chang d to a high protein diet containing 150 Gm of protein daily in the form of meat and li er by mouth Within a week a remarkabl impro ement in his general condition and hematologic stat occurred Within three months the patient gained nearly 4 stone in weight and his blood count and blood picture were restored to normal Of particular interest was the finding that free hydrochloric acid was again pres nt in his gastri juice Contact was kept with this patient for many years and it was found that th complet clinical and hematologic remission was maintained

This case represents a perfect example of a refractory megaloblastic anemia associated with the sprue syndrome which failed to respond to large quantities of crude potent liver extract given parenterally and showed a dramatic improvement when given liver by mouth

During the next ten years I occasionally encountered patients with the classic pernicious anemia blood picture who were refractory to parenteral liver therapy but who responded to liver given orally The problem of refractoriness was brought into prominence during an investigation which was conducted in Edinburgh into cases of pernicious anemia of pregnancy In this group of megaloblastic anemias we found that refractoriness to potent liver extracts given parenterally is not uncom mon The results of the investigation were published in 1942 (Davidson Davis and Innes) Of 16 cases with a classic megaloblastic marrow 10 were refractory to liver extracts given parenterally Shortly after this investigation our attention was attracted to the megaloblastic anemias associated with the sprue syndrome (tropical sprue and idiopathic steatorrhea) and here again we found patients who were either completely or partially refractory to potent liver extracts given paren terally In addition to cases of refractory megaloblastic anemia associated with pregnancy and the puerperium and the sprue syndrome we also encountered cases of refractory anemia whose etiology was completely obscure and to this group we gave the name idiopathic refractory megaloblastic anemia and it is with this group that this paper is particularly concerned

This short introductory note regarding our clinical investigations into refractory megaloblastic anemias over many years is given with the object of indicating why we desired to find a therapeutic agent which would be effective and why we be lieved that this product could be produced from liver which had not been submitted to a process of chemical purification for parenteral therapy

The first step in this investigation consisted of predigesting liver with the enzyme papain at a pH of 5 6 thus avoiding the danger of destruction of active principles

by exposure to acid or alkaline conditions The product obtained was a light brown powder completely soluble in water The name proteolysed liver was selected for descriptive purposes Since the walls of the liver cells had been completely disrupted it appeared likely that a high proportion of water soluble constituents would be liberated and hence retained in the final product and that other active principles present as a protein complex would be set free and so be rendered available for immediate absorption Clinical tests made with a 70 per cent alcohol soluble fraction of liver before and after digestion with papain supported this conclusion

It was estimated that 1 oz of proteolysed liver was derived from 6 oz of raw wet liver The material which has since been marketed under the trade name Hepamino was first tested on 5 cases of classic Addisonian pernicious anemia and produced a dramatic response in all instances in a daily dose of $\frac{1}{4}$ to $\frac{1}{2}$ oz A report on the method of preparation and its clinical trial was published in 1943 (Davis Davidson Riding and Shaw) During the next two years work was extended to testing the preparation in cases of refractory megaloblastic anemia Thus in 1944 we described the remarkable results produced in 4 cases of idiopathic megaloblastic anemia and in 1 case of refractory megaloblastic anemia of pregnancy (Davis and Davidson) We also noted its therapeutic failure in cases of macrocytic anemia with a normoblastic marrow

We suggested as a provisional hypothesis that While failure of maturation of the megaloblasts in the great majority of megaloblastic anemias is due to deficiency of the liver principle of Castle present in fractionated liver extracts in refractory megaloblastic anemias it results from an additional deficiency consequent on a failure in production or absorption of some unknown factor which is present in adequate amount and assimilable form in proteolysed liver and presumably also in whole liver In the same paper we discussed the possible nature of this factor and came to the conclusion that it was unlikely to be a mineral an amino acid or any of the vitamins available at that time for clinical use We suspected that it might be folic acid or biotin both of which were known to be present in considerable quantities in liver From our assessment of the position we felt that folic acid was most likely to be the missing factor and accordingly in 1944 we wrote to Dr Riding of Evans Medical Supplies Ltd asking him to make a preparation of folic acid for clinical trial in refractory megaloblastic anemias The folic acid fraction sent to us for this purpose consisted of material precipitated by 70 per cent alcohol from a watery extract of liver This fraction is discarded in the manufacture of parenteral liver extracts as it has been repeatedly shown to be impotent therapeutically Nevertheless it is in this fraction that most of the folic acid in liver is stated to occur as determined by biologic assay As was to be expected from previous clinical experience this fraction was found to be impotent when fed in daily doses of 1 oz to patients with pernicious anemia Unfortunately at that time no suitable case of refractory megaloblastic anemia was available on which to try the extract These results suggested that if folic acid was present in the nonproteolysed 70 per cent alcoholic precipitate of liver it existed in some conjugated form which could not be utilized by a patient with pernicious anemia or alternatively that

the content of folic acid in the test dose of extract was insufficient The next step taken was to submit the 70 per cent alcoholic precipitate of liver to papain digestion and see whether this would lead to the liberation of some hematinic factor which was not available in the nonproteolysed 70 per cent alcoholic precipitate Three cases of Addisonian pernicious anemia were treated with this proteolysed fraction Of these 1 case responded moderately well and 2 failed to show any response The single success achieved suggested that as a result of enzymic digestion some potent material had been liberated but that the amount so liberated was insufficient to produce satisfactory results From an assay of folic acid in proteolysed liver carried out at a later date this supposition was almost certainly correct Accordingly we decided to obtain a more potent source of folic acid and were in the process of investigating measures to achieve this object when the synthesis of folic acid was announced by Angier et al (1945) and its clinical and hematologic effects in megaloblastic anemias were published by Spies et al (1945) Through the courtesy of Messrs Lederle and Dr Spies we were fortunate in obtaining adequate supplies of folic acid and have thus been able to confirm the observations made by Spies and other workers in America in regard to its effectiveness in all forms of megaloblastic anemia (Davidson and Girdwood 1946 1947) Of particular interest to u was the determination of the smallest dose of synthetic folic acid which would produce a hematologic response in pernicious anemia as this was obviously a matter closely related to the problem of what constituent in proteolysed liver was responsible for its therapeutic activity in megaloblastic anemias refractory to potent parenteral liver extracts In this connection we should mention that of 5 cases of pernicious anemia treated with 2 5 mg of folic acid daily all responded satisfactorily Of 5 cases given 1 mg daily 1 gave an excellent response 2 a moderate response and 2 gave no response The last 2 cases subsequently responded well to a daily dose of 2 5 and 5 0 mg respectively The problem of the minimal effective dose of folic acid is referred to again in the discussion

Idiopathic Refractory Megaloblastic Anemia

During the past six years more than 450 cases of macrocytic anemia have been submitted to a full clinical and hematologic examination including sternal biopsy in the wards and blood clinics under my charge Approximately 75 cases out of this group were found to be cases of macrocytic anemia with a normoblastic marrow and need not be considered in this paper They included many examples of the sprue syndrome and cases of chronic hepatitis aplastic and hypoplastic anemia macrocytic hemolytic anemia and aleukemic leukemia Three hundred and fourteen cases were diagnosed as classical pernicious anemia and all responded satisfactorily to parenteral liver therapy proteolysed liver or folic acid In addition parenteral liver therapy was effective in 12 cases of megaloblastic anemia associated with pregnancy and the sprue syndrome Lastly there were 59 cases of megaloblastic anemia refractory to potent parenteral liver extracts Since a description has been given in our previous publications of cases associated with pregnancy and the puerperium and with the sprue syndrome it has been decided merely to illustrate representative cases of these groups with graphs showing the effect of treatment

FIG 1 A woman aged 3, with refractory megaloblastic anemia associated with idiopathic steatorrhea Response to folic acid (See Case History 2)

FIG 2 Male aged 32 with refractory megaloblastic anemia associated with tropical sprue Response to folic acid

with proteolysed liver and folic acid (figs 1 2 and 3) and confine our observations essentially to the group which we have called idiopathic refractory megaloblastic anemia

A patient is placed in this group only if the cause of the megaloblastic anemia cannot be ascribed to direct dietary deficiency pregnancy or the puerperium mal absorption from the gastrointestinal tract or hepatic disease It is obvious that the more thorough is the investigation and the more prolonged the period of observa tion the fewer will be the cases which will be classified as idiopathic This point is well illustrated by the following 2 case histories

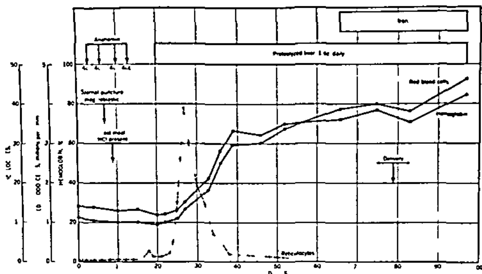


Fig 3 * Refractory megaloblastic anemia associated with pregnancy Response to proteolysed liver

CASE HISTORY 1

F st admission A man age 61 He gave a t vo years history of eakness breathlessness and anemia but there was no history of diarrhea of paresthesia of unsteadiness in walking or of pain in the tongue His diet had been satisfactory A diagnosis of pernicious anemia vas made in a neighboring hospital and the patient received 24 cc of a potent purified liver extract (Anahaemin) during a p-riod of four weeks The pat ent s condition continued to deteriorate so he was transferred to our clinic for further investiga tion and treatment

When admitted the patient was very weak His blood figures v ere as follows Hb 28 p-r cent R B C 950 000 per cu mm W B C 5 200 per cu mm P C V 13 0 per cent M C V 136 8 cu microns M C H C 30 0 per cent reticulocytes 2 2 per cent C I 1 5 The marrow was megaloblastic and a test meal showed that free hydrochloric acid was present No other abnormality w s found and at no time during the period of the first admission to our wards did the patient suffer from looseness of the bowels He as treated with 20 mg of folic acid daily and this resulted in a reticulocyte peak of 18 2 per cent a rapid gain in red cells and transformation of the marro v to the normoblastic st te E entually the red cell count reached a level of 5 million A diagnosis of idiopathic refractory meg loblastic anemia as made

* Figures 3 and 4 previously appeared in Pernicious Anemia and Other Megaloblastic Anemias L S P Dav dson and L J Davis in *Advances in Int rn l M d ci e* New York Intersci nce Publi h rs Inc 1947 vol II pp 481 547 Reproduced by permi sion of the publi hers

Second admission The patient returned to hospital a year later because his ankles were painful and swollen He had been having repeated courses of folic acid and the red cell count was moderately satisfactory being 4 110 000 per cu mm Examination of the stools revealed that he was now passing two large motions daily and these were pale greasy and bulky A fat balance test was carried out and this showed that the patient was absorbing only 59 per cent of ingested fat (normal 90 to 95 per cent) This clearly indicated that the patient was suffering from a malabsorption syndrome and accordingly the diagnosis was revised to that of idiopathic steatorrhea

CASE HISTORY 2

First admission A woman age 37 Admitted to hospital in March 1944 when she was five months pregnant Her hemoglobin was then 56 per cent and red cells 2 050 000 per cu mm The bone marrow was megaloblastic A test meal showed the presence of free hydrochloric acid A diagnosis of pernicious anemia of pregnancy was made She failed entirely to respond to 4 cc of Anahaemin given intramuscularly but responded to proteolysed liver an increase of red cells of one million per cu mm occurring in twenty days The patient was then discharged from hospital but owing to difficulty in obtaining proteolysed liver she did not continue treatment

Second admission She was readmitted in April 1946 with a history of weakness and of intermittent diarrhea of a fatty type a fat balance test showed the percentage absorption to be 75 per cent A test meal again showed the presence of free hydrochloric acid She had never been abroad and the dietetic history was normal No antianemic treatment had been given for eighteen months before the commencement of folic acid therapy At the start of folic acid therapy her blood findings were as follows Hb 40 per cent red cells 1 370 000 per cu mm white cells 7 800 per cu mm P C V 19 0 per cent M C V 138 7 cu microns M C H C 28 9 per cent reticulocytes 3 5 per cent C I 1 5

The reticulocyte response and the rise in red cells over a therapeutic period of 14 21 and 28 days reached the standards demanded by the U S P Board (see fig 1)

A diagnosis of idiopathic steatorrhea was mad

These case histories together with others which we do not think it is necessary to elaborate suggest that a failure in absorption from the alimentary tract may be a primary fault in some cases of idiopathic megaloblastic anemia In a few of these patients the poor bodily build and lack of development of the skeleton suggested previous malabsorption from the gastrointestinal tract though no history of diarrhea could be obtained at any time from infancy up to the presenting illness The absence of such a history may have little significance since we have clearly demonstrated in the sprue syndrome that fat absorption may remain grossly defective although diarrhea is absent and the patient s general health is good either as a result of folic acid therapy or from spontaneous remission (Davidson Girdwood and Innes 1947) Other possible causes of alimentary dysfunction which are worthy of consideration are an abnormal intestinal flora or chemical or enzymic secretory defects which destroy the antianemic factor or fail to liberate it from its bound form in natural foods

Some of the clinical and hematologic features of the group of cases labelled idiopathic refractory megaloblastic anemia are given in table 1 The patients were of both sexes and their ages ranged from 12 to 76 years The chief complaint in all cases was weakness and breathlessness on effort and physical examination usually revealed nothing of importance other than the signs and effects of severe anemia Acute glossitis and objective signs of involvment of the central nervous system were absent Chronic glossitis was frequently noted The liver spleen and lymphatic glands were not enlarged The blood picture and the bone marrow were

identical with that seen in Addisonian pernicious anemia at corresponding levels of anemia except in Cases 16 17 18 (see table 1) In those cases with histamine fast achlorhydria the differential diagnosis from pernicious anemia was impossible

TABLE 1 —*Cases of Idiopathic Refractory Megaloblastic Anemia*

Case No	Sex	Age	Free HCl	Before treatment		Treatment given	Initial hematologic response to treatment
				Hb %	Red cells (mill / cu mm)		
1	M	46	Absent	28	1 17	Intensive liver iron ascorbic acid transfusion	Slow and delayed
2	M	20	Present	23	0 88		Slow and delayed
3	F	55	Absent	18	0 77		Slow and delayed
4	F	51	Absent	31	1 31		Slow and delayed
5	F	41	Present	22	0 86		Slow and delayed
6	F	34	Absent	18	0 75		Slow and delayed
7	F	45	Absent	32	1 91		Slow and delayed
8	F	34	Absent	28	0 89		Slow and delayed
9	F	51	Absent	27	1 34		Slow and delayed
10	M	14	Absent	44	1 80	Proteolysed liver	Prompt
11	M	12	Present	32	1 13	Proteolysed liver	Prompt
12	F	61	—	42	1 [illegible]4	Proteolysed liver	Prompt
13	F	56	Absent	30	1 16	Proteolysed liver	Prompt
14							
1st admission	M	46	Present	36	1 24	Anahaemin	Slow and unsustained
2nd admission			Present	28	1 2	Proteolysed liver	Prompt
15	M	21	Present	46	1 [illegible]	Proteolysed liver	Prompt
16	F	[illegible]6	Absent	22	0 97	Proteolysed liver	Moderate response
17	F	65	—	26	0 98	Proteolysed liver & anahaemin	Moderate response
18	M	58	Present	38	1 65	Proteolysed liver	Moderate response
19	F	72	—	32	1 13	Proteolysed liver	Moderately rapid
20	M	66	Present	45	1 82	Proteolysed liver	Prompt
21							
1st admission	M	60	Absent	50	1 93	Proteolysed liver	Prompt
2nd admission			Absent	58	1 99	Folic acid	Prompt
22	F	52	Absent	34	1 06	Folic acid	Prompt
23	F	70	Absent	44	1 93	Folic acid	Prompt
24	F	63	Present	52	1 78	Folic acid	Prompt
25	M	53	Absent	38	1 28	Folic acid	Prompt

Cases 16 17 18 These 3 cases had a dimorphic bone marrow Although some typical megaloblasts were present the majority of the erythroblasts were normoblasts or cells intermediate in appearance between megaloblasts and normoblasts

Case 9 Owing to vomiting this patient was initially unable to take adequate quantities of proteolysed liver

until their failure to respond to parenteral injections of potent liver extract was discovered In other cases the presence of free hydrochloric acid in the gastric juice was a point of exceptional diagnostic importance Such cases would conform to the

Second admission The patient returned to hospital a year later because his ankles were painful and swollen He had been having repeated courses of folic acid and the red cell count was moderately satisfactory being 4 110 000 per cu mm Examination of the stools revealed that he was now passing two large motions daily and these were pale greasy and bulky A fat balance test was carried out and this showed that the patient was absorbing only 59 per cent of ingested fat (normal 90 to 95 per cent) This clearly indicated that the patient was suffering from a malabsorption syndrome and accordingly the diagnosis was revised to that of idiopathic steatorrhea

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First admission A woman age 37 Admitted to hospital in March 1944 when she was five months pregnant Her hemoglobin was then 56 per cent and red cells 2 050 000 per cu mm The bone marrow was megaloblastic A test meal showed the presence of free hydrochloric acid A diagnosis of pernicious anemia of pregnancy was made She failed entirely to respond to 4 cc of Anahaemin given intramuscularly but responded to proteolysed liver an increase of red cells of one million per cu mm occurring in twenty days The patient was then discharged from hospital but owing to difficulty in obtaining proteolysed liver she did not continue treatment

Second admission She was readmitted in April 1946 with a history of weakness and of intermittent diarrhea of a fatty type a fat balance test showed the percentage absorption to be 75 per cent A test meal again showed the presence of free hydrochloric acid She had never been abroad and the dietetic history was normal No antianemic treatment had been given for eighteen months before the commencement of folic acid therapy At the start of folic acid therapy her blood findings were as follows Hb 40 per cent red cells 1 370 000 per cu mm white cells 7 800 per cu mm P C V 19 0 per cent M C V 138 7 cu microns M C H C 28 9 per cent reticulocytes 3 5 per cent C I 1 5

The reticulocyte response and the rise in red cells over a therapeutic period of 14 21 and 28 days reached the standards demanded by the U S P Board (see fig 1)

A diagnosis of idiopathic steatorrhea was made

These case histories together with others which we do not think it is necessary to elaborate suggest that a failure in absorption from the alimentary tract may be a primary fault in some cases of idiopathic megaloblastic anemia In a few of these patients the poor bodily build and lack of development of the skeleton suggested previous malabsorption from the gastrointestinal tract though no history of diarrhea could be obtained at any time from infancy up to the presenting illness The absence of such a history may have little significance since we have clearly demonstrated in the sprue syndrome that fat absorption may remain grossly defective although diarrhea is absent and the patient s general health is good either as a result of folic acid therapy or from spontaneous remission (Davidson Girdwood and Innes 1947) Other possible causes of alimentary dysfunction which are worthy of consideration are an abnormal intestinal flora or chemical or enzymic secretory defects which destroy the antianemic factor or fail to liberate it from its bound form in natural foods

Some of the clinical and hematologic features of the group of cases labelled idiopathic refractory megaloblastic anemia are given in table 1 The patients were of both sexes and their ages ranged from 12 to 76 years The chief complaint in all cases was weakness and breathlessness on effort and physical examination usually revealed nothing of importance other than the signs and effects of severe anemia Acute glossitis and objective signs of involvement of the central nervous system were absent Chronic glossitis was frequently noted The liver spleen and lymphatic glands were not enlarged The blood picture and the bone marrow were

identical with that seen in Addisonian pernicious anemia at corresponding levels of anemia except in Cases 16 17, 18 (see table 1) In those cases with histamine fast achlorhydria the differential diagnosis from pernicious anemia was impossible

TABLE 1 —*Cases of Idiopathic Refractory Megaloblastic Anemia*

Case no	Sex	Age	Free HCl	Before treatment		Treatment given	Initial hematological response to treatment
				Hb %	Red cells (mill / cu mm)		
1	M	46	Absent	28	1 1[illegible]	Intensive liver iron ascorbic acid transfusion	Slow and delayed
2	M	10	Present	23	0 88		Slow and delayed
3	F	55	Absent	18	0 77		Slow and delayed
4	F	51	Absent	31	1 31		Slow and delayed
5	F	41	Present	22	0 86		Slow and delayed
6	F	34	Absent	18	0 75		Slow and delayed
7	F	45	Absent	32	1 91		Slow and delayed
8	F	34	Absent	28	0 89		Slow and delayed
9	F	51	Absent	27	1 34		Slow and delayed
10	M	14	Absent	44	1 80	Proteolysed liver	Prompt
11	M	12	Present	32	1 13	Proteolysed liver	Prompt
12	F	61	—	42	1 74	Proteolysed liver	Prompt
13	F	56	Absent	30	1 16	Proteolysed liver	Prompt
14							
1st admission	M	46	Present	36	1 24	Anahaemin	Slow and unsustained
2nd admission			Present	28	1 2	Proteolysed liver	Prompt
15	M	21	Present	46	1 77	Proteolysed liver	Prompt
16	F	[illegible]6	Absent	22	0 97	Proteolysed liver	Moderate response
17	F	65	—	[illegible]6	0 98	Proteolysed liver & anahaemin	Moderate response
18	M	58	Present	38	1 65	Proteolysed liver	Moderate response
19	F	72	—	32	1 13	Proteolysed liver	Moderately rapid
20	M	66	Present	45	1 82	Proteolysed liver	Prompt
21							
1st admission	M	60	Absent	50	1 93	Proteolysed liver	Prompt
2nd admission			Absent	58	1 99	Folic acid	Prompt
22	F	52	Absent	34	1 06	Folic acid	Prompt
23	F	70	Absent	44	1 93	Folic acid	Prompt
24	F	63	Present	52	1 78	Folic acid	Prompt
25	M	53	Absent	38	1 28	Folic acid	Prompt

Cases 16 17 18 These 3 cases had a dimorphic bone marrow Although some typical megaloblasts were present the majority of the erythroblasts were normoblasts or cells intermediate in appearance between megaloblasts and normoblasts

Case 19 Owing to vomiting this patient was initially unable to take adequate quantities of proteolysed liver

until their failure to respond to parenteral injections of potent liver extract was discovered In other cases the presence of free hydrochloric acid in the gastric juice was a point of exceptional diagnostic importance Such cases would conform to the

Fig 4 Idiopathic refractory megaloblastic anemia in a 12 year old girl Response to proteolysed liver

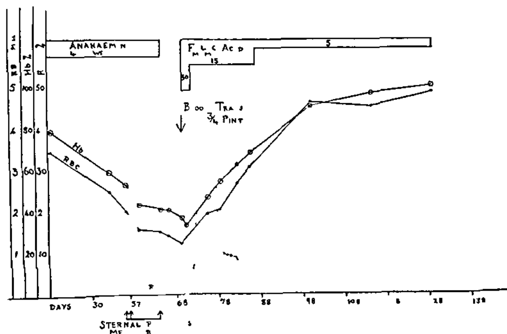


Fig 5 Idiopathic refractory megaloblastic anemia in a man aged 53 Response to folic acid

diagnosis of achrestic anemia as defined by Israels and Wilkinson (1936 1940) When the disease occurs in patients below the age of 30 and is not associated with pregnancy suspicion should be aroused that the case is not one of classic pernicious anemia Likewise if the state of nutrition is unsatisfactory and the skeleton poorly developed a malabsorption syndrome should be suspected As has already been emphasized the patients were only placed in the idiopathic group when search for a cause for the anemia had failed to establish any satisfactory etiological explanation With regard to treatment it should be noted in table 1 that cases 1 to 9 were under our care prior to the advent of proteolysed liver and folic acid They had a prolonged and dangerous illness lasting weeks or months and many would have succumbed if life had not been supported by repeated blood transfusions while intensive treatment with parenteral liver extract iron and vitamins was continued Cases 10 to 21 were treated with proteolysed liver and cases 21 to 25 with folic acid Case 21 was treated successfully in his first relapse with proteolysed liver A second relapse resulting from cessation of treatment responded excellently to folic acid In contrast to cases 1 to 9 whose response to treatment was slow and prolonged cases 10 to 25 responded to proteolysed liver or folic acid in a manner comparable to that obtained in pernicious anemia with parenteral liver extract therapy Representative examples of these cases are shown in figures 4 and 5

Discussion

The parenteral injection of chemically purified liver extracts and the oral administration of liver liver extracts proteolysed liver and folic acid produce identical effects in transforming the megaloblastic bone marrow of Addisonian pernicious anemia into the normoblastic state as has been clearly demonstrated by studies of the bone marrow by ourselves and other workers It may therefore be assumed that some common factor is responsible for this effect Since daily doses of 1 mg of synthetic folic acid will accomplish this result it appears likely that folic acid itself is the maturation factor or plays some essential role in the final stage of the process of maturation Since purified liver extracts for parenteral therapy are practically devoid of folic acid it seems not unreasonable to suppose that they produce a transformation of the bone marrow through their ability to liberate free folic acid from its conjugated state Hence it may be postulated that in pernicious anemia the inability of the stomach to produce Castle s intrinsic factor leads to a failure in the production of an interaction product whose function is to liberate free folic acid There appears to be no failure in the absorption of conjugated folic acid since an immediate response is obtained to the injection of purified liver extract even when the patient has been partaking of an unsatisfactory diet for long periods

In contrast the refractory megaloblastic anemias may be considered to be due to a failure in the supply of conjugated folic acid since no response occurs to the injection of large doses of potent purified liver extract This failure of supply may result from direct nutritional deficiency as occurs particularly in tropical countries such as India (Lucy Wills 1931) and the anemia may be partially or completely refractory to large doses of purified liver extract given parenterally Other cases can be explained on the basis of a malabsorption syndrome as is typically seen in tropical

sprue and idiopathic steatorrhea (see figs 1 and 2) As has already been noted some of these cases may not be recognized because a failure in absorption can occur in the absence of diarrhea In other cases the possibility exists that abnormalities in the intestinal flora or chemical and secretory changes in the alimentary tract may destroy folic acid or make it unavailable to the body in some way as yet unknown

Lastly the liver plays an important role in the storage and possibly the final synthesis of the liberating factor formed from the interaction of Castle s intrinsic and extrinsic factors It may also be of importance in the storage and liberation of free folic acid Accordingly it is not surprising that in severe chronic disease of the liver a megaloblastic anemia is occasionally found In our experience however, the macrocytic anemia of hepatic disease is accompanied much more frequently by a normoblastic marrow reaction *Accordingly it may be concluded that a deficiency* of conjugated folic acid can result from a variety of causes some of which are known while others can merely be suspected The resulting megaloblastic anemia will be partially or completely refractory to parenteral liver extracts depending on the relative degree of deficiency of conjugated folic acid All types of megaloblastic anemia respond to the administration of free folic acid This is not surprising since by giving free folic acid the need for an interaction to take place between the liberating factor contained in purified liver extracts and conjugated folic acid contained in food is circumvented What is surprising is the dramatic therapeutic effect produced by the oral administration of folic acid in the malabsorption syndromes such as sprue We can only assume that the capacity to absorb different substances in this syndrome varies greatly Thus absorption of fat appears to be particularly poor while the absorption of free folic acid must be nearly perfect since a daily dose of 5 mg or less will produce the most dramatic clinical and hematologic improvement in sprue cases with a megaloblastic anemia

Lastly it is necessary to consider why proteolysed liver is usually as effective in the treatment of refractory megaloblastic anemia as is free folic acid

Proteolysed liver contains the liberating factor present in chemically purified liver extracts for parenteral use Experience has shown however that oral treatment with whole liver is very much less effective than parenteral treatment with liver extract derived from an equivalent amount of liver

The therapeutic effects of proteolysed liver in refractory megaloblastic anemia cannot therefore be ascribed to its content of this factor Proteolysed liver is a rich source of amino acids readily available for absorption because of the predigestion of liver protein with papain We have treated cases of pernicious anemia with a papain digest of beef protein without any response and hence do not think it likely that the therapeutic activity of proteolysed liver depends on its content of amino acids It is possible however that liver contains some amino acid in high concentration which is necessary for the maintenance of normoblastic blood formation No evidence of this hypothesis is however available Supplementing the diet with individual amino acids such as methionine and choline has not been found effective in the treatment of the megaloblastic anemias Proteolysed liver is a rich source of many vitamins especially of the vitamin B complex including folic acid The question therefore arises whether the therapeutic effects of proteolysed liver can be

ascribed to its content of folic acid Before this question can be answered it would be necessary to undertake an assay of its folic acid content by the biologic methods at present in use Opinion appears to differ widely among experts whom we have consulted in regard to the accuracy of such methods when used for the estimation of folic acid in foods and tissues The only figures which we have available have been supplied to us by Dr Riding of Evans Medical Supplies Ltd who found an average figure of 0 8 mg of folic acid per oz of proteolysed liver This quantity approaches the lowest amount of synthetic folic acid which we have found to be effective in the treatment of Addisonian pernicious anemia As previously noted of 5 cases given 1 mg a day only 1 gave an optimal response 2 showed no response and 2 showed a moderate response In contrast the first 5 cases of pernicious anemia treated with proteolysed liver in doses of less than ½ oz daily (folic acid content 0 4 mg) all showed an optimal response It may be safely concluded that the therapeutic effect of free folic acid must have been augmented by other hematinic principles contained in the preparation e g the liberating factor and possibly other members of the vitamin B complex The lowest dose of folic acid which we have used for the treatment of refractory megaloblastic anemia is 2 5 mg daily and this dose was effective in the only case in which it was tried hence we are unable to state what is the minimal effective therapeutic dose in this group of anemias Even if it be assumed that it is in the region of 1 mg daily the results achieved by 1 oz of proteolysed liver daily in refractory megaloblastic anemia were superior to that produced by 1 mg daily of synthetic folic acid in pernicious anemia Accordingly we feel that the beneficial effects of proteolysed liver in refractory megaloblastic anemias cannot be explained solely on its content of folic acid nor on its content of the liberating factor since large amounts of purified liver extract given parenterally are ineffective

The superior efficiency of orally administered liver or proteolysed liver to liver extract given parenterally in refractory megaloblastic anemia can be explained on one or other of the following hypotheses (1) That some interaction takes place in the gastrointestinal tract between the ingested liver preparation and gastrointestinal enzymes which leads to a potentiation of hemopoietic factors already present or (2) that liver and proteolysed liver contain some essential hemopoietic principles including folic acid and possibly other members of the B_2 complex which are removed or destroyed in the chemical processes used in the manufacture of liver extracts for parenteral injection

Additional support for this latter view is suggested by the following observations We have many cases of tropical sprue and idiopathic steatorrhea who persistently have a moderate degree of macrocytic anemia which is entirely resistant to parenteral liver therapy The bone marrow presents a picture which is mainly normoblastic but many erythroblasts have an appearance intermediate between a megaloblast and a normoblast The failure of parenteral liver therapy and iron to restore the marrow picture to normal and the persistence of a macrocytic anemia clearly indicate a lack of some additional hematinic factor The hope that this factor would be folic acid was not realized as can be clearly seen in the

protocols of our cases published in 1946 (Davidson, Girdwood and Innes) Proteolysed liver also was found to be ineffective in some of these cases of refractory macrocytic anemia associated with the sprue syndrome In 2 or 3 cases, however, it caused a considerable increase in the blood level after parenteral liver therapy and folic acid by mouth had failed In such cases we concluded that proteolysed liver contained some additional hematinic principle whose composition was still unknown An investigation into the nature of this principle is proceeding Our therapeutic program in operation at the present time is based on the investigations detailed above Our practice is to treat all cases of megaloblastic anemia in the first instance with parenteral liver extracts Should the result be unsatisfactory we prescribe folic acid If this fails to restore the blood picture to normal we give 1 oz of proteolysed liver daily By this means we are able to restore the blood picture qualitatively and quantitatively to normal in the great majority of cases of megaloblastic anemia of all types

Summary

1 Fifty nine cases of megaloblastic anemia refractory partially or completely to potent liver extracts given parenterally have been investigated in Edinburgh during the past six years Thirty four of these cases were associated with pregnancy the puerperium or the sprue syndrome No explanation of the cause of the megaloblastic anemia was discovered in the remaining 25 cases

2 The etiology clinical features and treatment of 25 cases of idiopathic refractory megaloblastic anemia are described Attention is directed to the excellent therapeutic effects produced by proteolysed liver or folic acid

3 The mechanisms involved in refractoriness to potent parenteral liver extracts are discussed

4 In certain cases of refractory megaloblastic anemia it is suggested that an unknown hematinic principle in addition to the liberating factor in purified parenteral liver extract and folic acid is required for the complete restoration of normoblastic blood formation

Acknowledgments

My thanks are due to many members of my staff who have helped in these investigations particularly to Professor L J Davis formerly lecturer in Medicine in the University of Edinburgh and to Dr Girdwood Grateful acknowledgment must also be made to Doctor Riding Medical Director of Evans Medical Supplies Ltd and his research chemists who were responsible for the preparation of proteolysed liver and the other fractions of liver mentioned above

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THE STUDY OF THE MYELOGRAM (BONE MARROW PUNCTURE) IN PERNICIOUS ANEMIA AND THE PROBLEM OF THE MEGALOBLAST

By JACQUES MALLARME M D

OF ALL the recent advances in medicine the conquest of pernicious anemia is one that should make pathologists proud Twenty five years ago Biermer s anemia was a pernicious anemia often fatal and which could not be cured by any treatment It is fair to acknowledge the brilliant work of Minot and Murphy, who conceived the idea of applying the liver treatment inaugurated by Whipple to pernicious anemia The results were such that since then patients with pernicious anemia do not die of the disease any more Simultaneously hepatotherapy has provided a convincing argument in favor of the specificity of the disease Confusion with other severe types of anemia has been avoided In addition to hepatotherapy other arguments have supported the concept of pernicious anemia as an autonomous disease entirely distinct from other types of anemia Among these arguments one of the most recent is based on the study of the myelogram in pernicious anemia

The cytology of the bone marrow was already known before the use of sternal punctures However postmortem examinations are vitiated by the onset of cadaveric changes in the tissues Therefore the sternal biopsy is indispensable to an understanding of the bone marrow This is why the technic of bone marrow biopsy introduced in 1929 by Arinkin provided a great impetus to the development of hematology This applies particularly to the study of pernicious anemia

Sternal puncture is a simple procedure carried out with a special needle containing a stilet which penetrates easily through the anterior table of the sternum local anesthesia is not necessary The bone marrow is aspirated in very small quantities if one wants to have a rich myelogram Reading of the myelogram is easy if the technic of preparation is adequate spreading—compression between two slides of a very small quantity of tissue preferably small fragments of bone marrow—double staining by May Grunwald Giemsa for one half hour The water used should have a neutral pH

Naegeli first described the erythroblasts which characterize pernicious anemia He called them *megaloblasts* and this term was responsible for much of the confusion that prevailed thereafter This confusion arose from the fact that Ehrlich and also Jolly gave the name megaloblast to certain types of very young erythroblasts which are basophilic and are found in any normal marrow These are not the megaloblasts which *are found in pernicious anemia These normal erythroblasts* are also called pronormoblasts basophil normoblasts or Naegeli s normoblasts

The megaloblasts found in pernicious anemia represent a special lineage of erythroblasts ultimately ending up in a special type of erythrocyte the megalocytes and having a characteristic morphology at each stage of the cytologic development

Paris France

As seen in the bone marrow smear the megaloblasts of pernicious anemia have a high optical density The youngest forms arising from the reticulum are the promegaloblasts (called erythrogones by certain authors) They have a histio blastic appearance with a grayish blue protoplasm more or less spread out not sharply limited with pseudopods and sometimes a protoplasmic connection with another promegaloblast The nucleu is enormous and the chromatin shows a fine lacy structure with numerous bluish nucleoli

Following the promegaloblasts come the basophilic megaloblasts which are frequently seen These cells are large with a big round nucleus centrally placed The chromatin has a fine skeinlike structure and is transparent The protoplasm is very basophilic has a variable thickness and is usually quite wide In some cases the basophilic megaloblast shows early eosinophilic granules

As maturation progresses the cellular anarchy becomes more manifest and the aspect of the megaloblast diverges more and more from that of the normoblast The nucleus remains large with a fine transparent chromatin which in places con denses into pearl like or blocklike structure The peripheral protoplasm becomes lighter in an irregular fashion and its maturation may lag behind or more often precede that of the nucleus

The fourth stage of the development is that of polychromatophilic megaloblast the asynchromism between nucleus and protoplasm becomes manifest a big nucleus may be found in conjunction with a small cytoplasm or a small nucleus in conjunc tion with a large cytoplasm The cytoplasm shows vivid colors purple or greenish with heterogeneous areas of variable shapes The nucleus still has a partly reticu lated structure and may undergo amitotic division or fragmentation which pro duces an aspect of a petalled flower The nucleus is still young as shown by its transparent and pearl like aspect But the tendency toward the formation of frag ments of the nucleus classifies the cell as an old type of cell The nuclear fragments appearing early in the cell are the future Jolly s bodies

The orthochromic megaloblasts have an orange color but may contain basophilic remnants in the form of basophilic areas or granules as in lead poisoning The nuc leus may be round and regular as the nucleus of the orthochromic normoblast but more often it is reniform or dumbell shaped or has the shape of a clover leaf The orthochromic megaloblasts vary in size and some may be rather small When they lose their nucleus they become remnants Cabot s rings and Jolly s bodies The megaloblasts in pernicious anemia often show atypical mitotic figures at all phases

In summary at all stages of the evolution the megaloblast is an abnormal dys trophic erythroblast resulting in the creation of a large erythrocyte This explains the anisocytosis poikilocytosis and megalocytosis which are the expression of a cellular dystrophy and not of a cellular immaturity since most cells observed in the blood smear of a case of pernicious anemia are completely mature cells

After a long period of discussion hematologists agree among themselves that the megaloblastosis belongs specifically to pernicious anemia I personally do not think that megaloblasts are observed in any anemia except pernicious anemia pro vided one adheres to the definition of the megaloblast as I have given it and one does not consider the megaloblast as a very young nucleated basophilic erythrocyte

Every time I have been invited to see so called megaloblasts in the myelograms from patients having blood disease but no pernicious anemia I have recognized that these were not megaloblasts While the megaloblast is specific for pernicious anemia there are also normoblasts and their proportion to the number of megaloblasts is variable They are seen in early cases of pernicious anemia Also they completely and rapidly replace the megaloblasts as soon as hepatotherapy is instituted This fact permits one to conclude that the megaloblastosis is conditioned by a deficiency of the hemopoietic factor of maturation of red cells which is provided by the liver

The generally accepted opinion proposed by Naegeli by Ferrata and most hematologists is that the megaloblast represents a revival of the embryonic erythrocyte of the first generation There is a certain morphologic analogy between the megaloblast and the nucleated erythrocyte of the fetus One arrives therefore at the conception as already advanced by Dameshek and Wilkinson and Israels that there are two types of hemopoiesis the normoblastic or adult type and the megaloblastic or embryonic type Their appearance or disappearance depends on the factor of Whipple if the maturation factor is deficient the fetal type of erythroblasts appears in the bone marrow but if the factor is administered the normal type of erythroblasts replaces the megaloblasts

My interpretation is a little different from what precedes The megaloblast does not appear as the result of a substitution of two erythroblastic lineages because such a substitution is never observed Rather than a substitution a real transformation occurs which changes the normal erythroblast into a megaloblast this morphologic transformation is caused by a deficiency of the maturation factor In other words the megaloblast is a normoblast suffering from a nutritional deficiency

This interpretation explains several particularities of the disease first of all the megaloblast may exhibit morphologic monstrosities in a varying degree according to the degree of the deficiency This is analogous to what is seen in dystrophies caused by endocrine or vitamin deficiencies In very advanced cases of pernicious anemia the megaloblasts are typical and numerous In incipient cases the erythroblasts are not very much different from normoblasts and there are intermediary forms between normoblasts and megaloblasts Finally when the treatment by liver injections is instituted there is a rapid transformation of the megaloblasts into normoblasts

The metaplasia from normoblast to megaloblast or vice versa always affects the young forms of the series proerythroblasts and basophilic erythroblasts This is why in cases of pernicious anemia in relapse the young basophilic cells are megaloblastic while the older cells are normoblastic As soon as the liver treatment is instituted the myelogram shows a normoblastic transformation of the basophilic erythroblasts without change in the polychromatophilic and orthochromic megaloblasts

Another proof of the existence of a nutritional dystrophy in pernicious anemia is provided by the aspect of the granulocytes and megakaryocytes of the bone marrow

The granulocytes show morphologic changes the myelocytes are very large and

pale looking with enormous nuclei The metamyelocytes have a ribbon shaped nucleus with pseudopods The polymorphonuclears have a polysegmented nucleus (up to 15 segments) giving the appearance of a knotted chord

The megakaryocytes also have a polysegmented nucleus all the cells formed in the bone marrow are affected in pernicious anemia *Biermer s disease is a dystrophic myelosis affecting the bone marrow as a whole and producing anemia neutropenia and thrombocytopenia* The morphologic changes resulting from the dystrophy are specific and constitute the basis for the diagnosis

From a scientific point of view the myelogram in pernicious anemia is very interesting because it constitutes an instance of a cytologic dystrophy which can be reversed by the administration of liver of Castle s factor of folic acid

One cannot help being struck by the analogy between the cytologic dystrophy of pernicious anemia and certain cellular alterations of malignant neoplastic tissues In both cases there is the same excessive proliferation the same cellular monstrosity the same cytoplasmic nuclear asynchronism the same young aspect of the nucleus same abnormal mitotic or amitotic cellular division

On the other hand hepatotherapy or folic acid treatment are very similar to vitamin therapy

At the present time therefore one can say without exaggerating too much that pernicious anemia is a nutritional deficiency and a disease rather akin to cancer in which the abnormal cellular proliferation is corrected by a chemically defined organic substance

PART II

HEMOLYTIC ANEMIA

13

STUDIES ON THE DESTRUCTION OF RED BLOOD CELLS * IV

THERMAL INJURY ACTION OF HEAT IN CAUSING INCREASED SPHEROIDICITY OSMOTIC AND MECHANICAL FRAGILITIES AND HEMOLYSIS OF ERYTHROCYTES OBSERVATIONS ON THE MECHANISMS OF DESTRUCTION OF SUCH ERYTHROCYTES IN DOGS AND IN A PATIENT WITH A FATAL THERMAL BURN

By THOMAS HALE HAM M D SHU CHU SHEN M D ELEANOR M FLEMING A B AND W B CASTLE M D

IN A PREVIOUS communication[1] observations were reported on the changes in blood and urine and on the kidney complications occurring in 14 patients with moderate or severe thermal burns 11 of whom showed hemoglobinuria The spherocytosis and increased osmotic fragility of the red blood cells observed in certain of these cases were considered to result directly from the heating of the circulating blood This paper reports detailed observations on the effect of heat on human erythrocytes and on the mechanism of destruction of heated dog red blood cells following their injection into the same animal Finally the characteristics of the red blood cells are reported in an additional case of a fatal thermal burn

Previous investigations have established certain fundamental facts concerning the effect of heating red blood cells in the test tube and in the animal as a result of thermal burns There is agreement [-7] with the original observation of Schultze[8] that the heating of blood in vitro from human subjects dogs cats and rabbits to temperatures of from approximately 52 to 65 C produces division and fragmentation of erythrocytes with the formation of spheroid forms of various sizes In anesthetized animals subcutaneous temperatures of from 51 to 65 C have been maintained for several minutes by scalding [9] by igniting turpentine on the skin [10] or by use of a hot iron [11] Furthermore it has been demonstrated [10, 12, 13] especially by von Lesser that fragmented erythrocytes occurred in the blood stream in burned animals in normal animals transfused with blood from a burned animal and in normal animals transfused with blood heated in vitro The abnormal erythrocytes disappeared rapidly from the animal s circulation with the development of hemoglobinemia and hemoglobinuria No hemolysins or agglutinins have been demonstrated in the plasma or serum of burned animals[5] or of burned patients [1] An increase in the osmotic fragility of the red blood cells was noted in burned animals by Silberman[3] and in human blood heated in vitro by Isaacs Brock and Minot [7] The latter investigators demonstrated that immature erythrocytes of both normal and pathologic human blood divided less readily than mature erythrocytes when heated to 55 C More recently hemoglobinemia hemoglobinuria fragmentation of erythrocytes and increase in the osmotic fragility of red cells have been reported in a series of cases of human thermal burns by Brown[14, 15] *and in animals subjected to*

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thermal injury by Moritz Henriques and Dutra[16] and by McLean Moritz and Roos [17] Hemoglobinemia and increased blood destruction have been observed as an early manifestation of severe burns in human subjects by Moore Peacock Blakely and Cope[18] and in burned animals by Olson and Necheles [19]

Methods

The methods employed in this investigation have been described in previous communications as follows determination of osmotic fragility of red blood cells[1] determination of mechanical fragility of red blood cells[20] estimation of the number of spherocytes in a stained smear[1] determination of hematocrit[21] quantitative estimation of hemoglobin in plasma and urine [22] [24] measurement of pH of blood and urine by glass electrode method measurement of methemoglobin and sulphemoglobin [25]

Effect of Heat on Erythrocytes

Changes in the shape osmotic fragility and volume of red cells were measured in 131 observations in defibrinated blood from 12 normal persons and from dogs The blood was exposed to temperatures of from 25 to 70 C maintained for periods of from two minutes to one hour All blood samples were heated usually in 5 or 6 cc amounts in soft glass test tubes of 13 mm diameter by 150 mm length For larger volumes pyrex Erlenmeyer flasks were employed Each blood sample was first placed in a water bath at 37 C until this temperature was attained Then the sample was immersed in a bath containing 30 liters of water which was agitated by a mechanical stirrer and maintained at constant temperature with an accuracy of ±0 05 C A precision thermometer was introduced directly into the blood sample as it was heated and the temperature recorded every fifteen seconds usually during gentle mixing with the thermometer The blood sample was heated from 37 C to a particular temperature in approximately 2½ minutes and maintained for a given time within ±0 05 C at the required temperature The sample was then removed from the bath and promptly cooled to 37 C in a water bath The rates of heating and cooling were approximately equal For the so-called rapid heating the water bath temperature was set 0 7 C above the final temperature desired for the blood sample It required from one to two minutes in each observation to reach the desired temperature at which time the blood was immediately removed and cooled as described above Unheated and heated samples were compared with respect to hemolysis osmotic fragility and shape of the red blood cells hematocrit pH methemoglobin sulphemoglobin and nonprotein nitrogen

The changes produced in the red blood cells appeared in the following order morphologic changes apparent increase in volume increase in osmotic and mechanical fragilities and finally hemolysis in the serum or plasma Temperatures up to 46 C for a period of one hour caused no demonstrable changes in the erythrocytes At temperatures from 47 to 50 C changes in the red blood cells occurred depending on the temperature and duration of heating At temperatures of from 51 to 65 C changes always occurred even when the sample was subjected to rapid heating Changes occurred in dog red cells similar to those observed in human red cells The various effects produced by heat are described separately below

A CHANGES IN MORPHOLOGY OF ERYTHROCYTES PRODUCED BY HEAT

The morphology of the red blood cells was studied in wet preparations made by diluting defibrinated blood with serum physiologic saline or Gower s solution and introducing one drop into a standard blood counting chamber or onto a glass slide covering it with a glass coverslip and sealing with vaseline In fixed preparations stained with Wright s stain the red cells were examined for changes in size and shape including the presence of spherocytes and target forms The diameter of the red blood cells before and after heating was measured in stained preparations by the Price Jones [8] method Because of the bizarre forms produced by heating these measurements serve only as an approximation

TABLE 1 —*Effect on Morphology and Osm tic Fragility f R d Blo d Cells Resulting f om Heating Normal H man Defibr nat d Bio d at 48 6 C for Increas g Periods of Time (see Figure 1)*

D tio f heat g t 48 6 C	App r t c eas h m t c it	H m ly alt b t g	Osmot c frag l ty f ed blood c ll Hemolys							Morpholog c observ t s o red blood ell					
			1	2.5	5	10	50	75	95	gme te l f rms	Sph ytes	Microcytes	Me n C rp s cul r Di m ter	St nd d D i t n f om Mean Di met r	C ff e t f V ri ati n (Di met r)
			To i ty a NaCl Cm												
	%	%									%	%	µ	µ	%
Unheated control	0	0	40	40	39	37	35	33	29	0	1	0	- 3	0 67	9 2
Rapid heating	+0 6	0 4	40	40	39	37	34	32	23	0 5	0 2	0	- 3	0 52	7 1
2 Minutes	+3 2	0	41	40	40	38	36	34	29	1	0 4	0			
5 Minutes	+5 8	0 4	40	40	39	37	35	33	30	5	1	0 6		*	
10 Minutes	+4 3	0 4	56	42	40	38	33	33	28	12	1 5	3			
30 Minutes	+6 7	0 7	79	76	68	59	40	36	29	4†	8†		5 5	1 0	18
60 Minutes	+10 0	0 4	82	78	76	63	44	40	30		Many†		5 4	0 82	15

Quantitative measurement was not possible
† Rough approximation

At temperatures of from 48 6 to 49 6 C it was possible to observe the slow progression of the morphologic changes in the erythrocytes A typical experiment *conducted with heating of defibrinated blood at a constant temperature of 48 6 C* for 2 5 10 30 and 60 minutes respectively is illustrated in table 1 and figure 1 The first discernible change produced by heat was the appearance of small bud like protrusions on an occasional erythrocyte In the next recognizable change many of the erythrocytes showed single or multiple buds usually connected by a broad base or a filament When completely disconnected the new elements formed small rounded or elongated structures containing various amounts of hemoglobin At this stage as illustrated in figure 1B and table 1 (2 and 5 minutes) there was no change in osmotic fragility of the red blood cells but apparent increases in hematocrit of 3 2 to 5 9 per cent respectively It is possible that the increase in hematocrit

thermal injury by Moritz Henriques and Dutra[16] and by McLean Moritz and Roos [1] Hemoglobinemia and increased blood destruction have been observed as an early manifestation of severe burns in human subjects by Moore Peacock Blakely and Cope[18] and in burned animals by Olson and Necheles [19]

Methods

The methods employed in this investigation have been described in previous communications as follows determination of osmotic fragility of red blood cells[1] determination of mechanical fragility of red blood cells[20] estimation of the number of spherocytes in a stained smear[1] determination of hematocrit[21] quantitative estimation of hemoglobin in plasma and urine [22] [24] measurement of pH of blood and urine by glass electrode method measurement of methemoglobin and sulphemoglobin [25]

Effect of Heat on Erythrocytes

Changes in the shape, osmotic fragility and volume of red cells were measured in 131 observations in defibrinated blood from 12 normal persons and from dogs The blood was exposed to temperatures of from 25 to 70 C maintained for periods of from two minutes to one hour All blood samples were heated usually in 5 or 6 cc amounts in soft glass test tubes of 13 mm diameter by 250 mm length For larger volumes pyrex Erlenmeyer flasks were employed Each blood sample was first placed in a water bath at 37 C until this temperature was attained Then the sample was immersed in a bath containing 30 liters of water which was agitated by a mechanical stirrer and maintained at constant temperature with an accuracy of ±0 05 C A precision thermometer was introduced directly into the blood sample as it was heated and the temperature recorded every fifteen seconds usually during gentle mixing with the thermometer The blood sample was heated from 37 C to a particular temperature in approximately 2½ minutes and maintained for a given time within ±0 05 C at the required temperature The sample was then removed from the bath and promptly cooled to 37 C in a water bath The rates of heating and cooling were approximately equal For the so-called rapid heating the water bath temperature was set 0 7 C above the final temperature desired for the blood sample It required from one to two minutes in each observation to reach the desired temperature at which time the blood was immediately removed and cooled as described above Unheated and heated samples were compared with respect to hemolysis osmotic fragility and shape of the red blood cells hematocrit pH methemoglobin sulphemoglobin and nonprotein nitrogen

The changes produced in the red blood cells appeared in the following order morphologic changes apparent increase in volume increase in osmotic and mechanical fragilities and finally hemolysis in the serum or plasma Temperatures up to 46 C for a period of one hour caused no demonstrable changes in the erythrocytes At temperatures from 47 to 50 C changes in the red blood cells occurred depending on the temperature and duration of heating At temperatures of from 51 to 65 C changes always occurred even when the sample was subjected to rapid heating Changes occurred in dog red cells similar to those observed in human red cells The various effects produced by heat are described separately below

ghosts and many polymorphic fragments varying in size from 1-2 microns down to innumerable minute particles approximately the size of bacteria showing active Brownian movement in wet preparation Microscopic examination of the erythrocytes in a wet preparation did not reveal Heinz Ehrlich bodies [27] [28]

B CHANGES IN OSMOTIC FRAGILITY OF ERYTHROCYTES PRODUCED BY HEAT

Coincident with the continued fragmentation of erythrocytes the osmotic fragility became significantly increased The production of spheroid erythrocytes was evident from inspection of wet preparations and from the decrease in diameter and dense staining of red blood cells in stained preparations As shown in table 1 (30 and 60 minutes) the increased osmotic fragility was also associated with a decrease in the mean diameter of the erythrocytes and an increase in the coefficient of variation of the diameters It should be emphasized that progressive fragmentation of the erythrocytes occurred without significant loss of hemoglobin unless the osmotic fragility of a portion of the population had increased to such an extent that hemolysis occurred in concentrations of sodium chloride of from 0 85 to 1 0 grams per cent Thus when the osmotic fragility was normal the free hemoglobin in the plasma was less than 1 per cent of that contained by the cells of the sample this was also observed in many instances when both fragmentation and fragility were greatly increased as shown in table 1 and in table 2 However with sufficient heating the osmotic fragility of the red cells could be so increased as to produce marked hemolysis of cells in serum or plasma Thus rapid heating at temperatures of from 55 to 60 C produced up to 5 per cent hemolysis at temperatures of from 62 to 65 C from 22 5 to 100 per cent hemolysis

In contrast to the morphologic changes in erythrocytes which were heterogeneous and difficult to evaluate quantitatively the changes in osmotic fragility produced by heating were definitive and readily measured as shown in figure 2 The change in osmotic fragility caused by a particular temperature and period of heating was remarkably reproducible for blood samples obtained at the same time or at different times from normal persons When different blood samples were heated for a fixed period of time a critical temperature was found above which an increase of from only 1 0 to 1 6 C produced a phase of rapidly increasing osmotic fragility as illustrated in figure 2 and table 2 Below this critical temperature range the osmotic fragility was always found to be normal For example rapidly increasing osmotic fragility values occurred for rapid heating between 50 6 and 51 6 C and for a period of sixty minutes of heating between approximately 47 and 48 6 C Moderate and similar increases in osmotic fragility were produced by the following temperatures and periods of heating at approximately 50 8 C by rapid heating and at approximately 49 2 49 48 6 48 4 and 48 C by 2 5 10 30 and 60 minutes heating respectively In order to illustrate the reciprocal relation between the effects of temperature and the duration of heating sufficient to produce a given increase in osmotic fragility these data were plotted in two ways in figure 3 The approximately straight line function between the reciprocal of the absolute temperature and the logarithm of the time (equivalent to the rate) to be seen in figure 3B suggests the conformity of enzyme reactions or chains of reactions with Ar

in this and subsequent experiments did not represent a true swelling of the erythrocytes but rather an inability to pack the malformed erythrocytes by centrifugation at 3000 r p m This apparent increase in hematocrit occurred simultaneously with morphologic alteration of the erythrocytes increased slightly with their progres

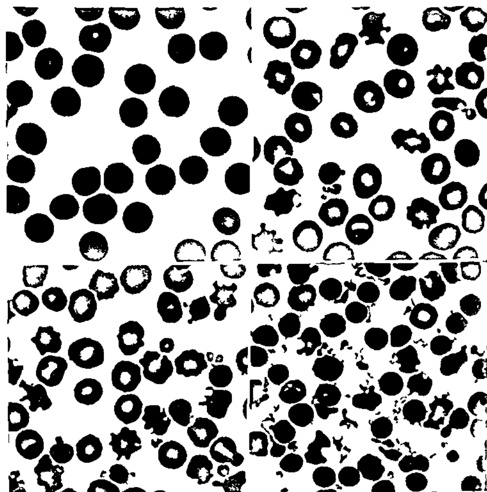


FIG 1 EFFECT OF HEAT ON MORPHOLOGY OF RED BLOOD CELLS

A upper left unheated human defibrinated blood Other samples heated at a temperature of 48 6 C B upper right for 5 minutes C lower left for 10 minutes D lower right for 30 minutes Stained blood films × 1000 (See table 1)

sive alteration and thereafter did not vary in parallel with the increase in osmotic fragility This phenomenon was not investigated further

The first increase in osmotic fragility occurred after still further fragmentation of erythrocytes and coincidentally with the appearance of significant numbers of densely staining red cells of various sizes which appeared spheroid in shape in wet preparations and densely stained in fixed smears as may be seen in figure 1C and table 1 (10 minutes) Continued fragmentation as illustrated in figure 1D resulted in the conversion of the majority of erythrocytes to spheroid cells occasional

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TABLE 2.—*Summary of Effect on Osmotic Fragility and Hemolysis of Red Blood Cells Resulting from Heating Normal Human Defibrinated Blood at Various Temperatures for Increasing Periods of Time*

Rapid heating

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
48.6	41	39	37	34	32	0.4
49.0	41	37	36	34	32	0
49.6	42	40	39	35	32	0.3
	42	40	39	35	32	0.3
50.0	44	40	39	36	33	0.3
50.6	44	40	39	35	32	0.6
51.0	77	68	58	36	33	1.2
	85	69	58	3	30	1.2
51.6	80	77	75	58	42	0.3
52.0	81	79	7	64	47	0.3
	85	9	77	67	58	0.1
53.0	8	80	78	69	55	1.2
54.0	83	9	77	63	48	1.2
55.0	85	79	76	56	43	0.7
	84	73	77	65	48	0.7
	8	82	80	67	52	1.4
56.0	85	80	78	60	46	1.3
57.0	85	79		5	42	1.9
58.0	87	80	77	58	42	2.1
59.0	94	86	81	58	45	3.3
60.0	>1	90	83	6	49	4.8
62.0	>1	>1	97	68	59	22.5
63.0	>1	92	84	65	54	17.0
65.0						Complete
70.0	Coagulated					

Heating for 2 minutes

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
40.2	44	40	39	35	34	0.2
45.0	42	40	39	35	34	0.5
48.0	42	40	38	34	33	0.4
48.6	41	40	38	36	34	0
49.0	52	40	39	34	31	0.1
49.6	80	74	68	38	33	0.6
50.0	84	79	7	68	53	0.8
	85	79	77	60	43	0.9
	82	78	76	59	41	0.1
51.0	87	83	81		58	1.5
52.0	87	82	79	68	55	1.6
52.6	89	83	80	69	56	1.5
	88	82	9	68	53	2.8
53.0	91	84	81	64	51	1.4
55.0	90	83	80	64	47	2.6
	1 0	91	88	70	58	2.3
	85	80	8	62	47	1.6
56.0	89	83	80	63	47	3.0
58.0	90	83	80	66	51	7.0
62.0						7.2
65.0	Coagulated					

Heating for 5 minutes

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
48.6	41	39	37	35	33	0.4
	41	39	37	33	31	0.8
49.0	73	50	40	33	30	0.4
49.6	80	76	75	59	44	0.7
50.0	85	78	76	68	54	0.9
52.6	91	84	81	0	58	3.0

Heating for 10 minutes

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
48.0	44	39	37	31	27	0.5
48.6	71	45	37	33	30	0.9
	56	40	38	35	33	0.4
	74	57	44	33	30	0.3
49.0	80	75	0	41	35	0.7
49.6	82	9	77	67	51	1.3
	84	82	79	68	52	1.0
	85	79	8	64	48	0.7
52.6	90	84	83	69	54	5.4

Heating for 30 minutes

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
47.0	46	40	39	33	33	0.6
47.6	44	40	39	35	34	0.2
48.0	47	41	39	35	34	0.5
48.6	80	69	59	41	37	0.4
	79	68	59	40	36	0
49.0	86	80	78	57	45	1.1
	86	80	78	63	49	1.3
52.6	>1	96	91	71	55	18.0

Heating for 60 minutes

Temperature of blood, °C	Osmotic fragility RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75	Hemolysis after heating, %
45.0	41	39	37	34	33	0.2
46.0	44	39	37	34	33	0
47.0	45	40	39	35	33	1.0
47.6	49	41	40	35	33	0.7
48.0	67	52	45	36	34	1.1
48.6	84	6	69	52	42	1.4
	82	6	63	44	40	0.4
49.0	90	83	80	59	47	3.0
52.6	>1	>1	91	6	60	47.0

Control observations on unheated blood

	Osmotic fragility of RBC Hemolysis 1 (Tonicity as NaCl Gm %)	5	10	50	75
Maximum	0.44	0.40	0.40	0.36	0.34
Minimum	0.39	0.36	0.35	0.31	0.30
Average	0.41	0.39	0.38	0.34	0.33

20 observations on 3 different normal subjects

thenius law [21] No attempt was made to interpret these data further Henriques[20] has made a mathematical analysis of experimental time temperature relationships for thresholds of epidermal injury

Observations were made of the changes in morphology and osmotic fragility produced by the heating of blood samples containing abnormally spheroid red cells from patients with chronic hypochromic anemia and sickle cell anemia respectively The results are summarized in table 3 In each instance experimental conditions were arranged so that the heating of the blood sample increased the osmotic fragility of the red cells to approximately the same final value The osmotic fragility of the red cells from the patient with congenital hemolytic jaundice was

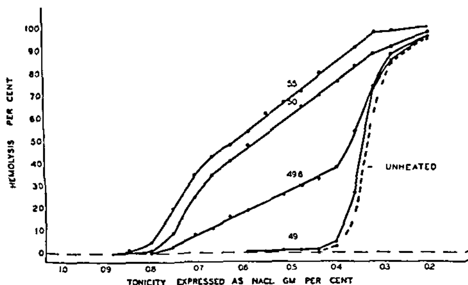


FIG 2 OSMOTIC FRAGILITY OF RED BLOOD CELLS OF SAMPLES OF HEATED NORMAL HUMAN DEFIBRINATED BLOOD

The individual curves represent effects of heating for 2 minutes at temperatures of 49 49 6 50 and 55 C respectively as indicated

of course already increased and before heating stained films showed 3 per cent of spherocytes With heating only a moderate degree of fragmentation and of decrease in mean corpuscular diameter was required to produce the observed increase in osmotic fragility On the contrary for the relatively discoid flat or target cells with initially decreased osmotic fragility the given degree of osmotic fragility was reached only after considerable morphologic change had appeared indicated by marked decrease in mean corpuscular diameter and increase in the coefficient of variation of the erythrocyte diameters Paradoxically the temperature required to produce the given degree of morphologic change was greater for the spheroid than for the discoid cells Thus blood from the patient with congenital hemolytic jaundice required rapid heating to 52 C that from the patient with sickle cell anemia to 50 6 C in order to produce the same final value for osmotic fragility

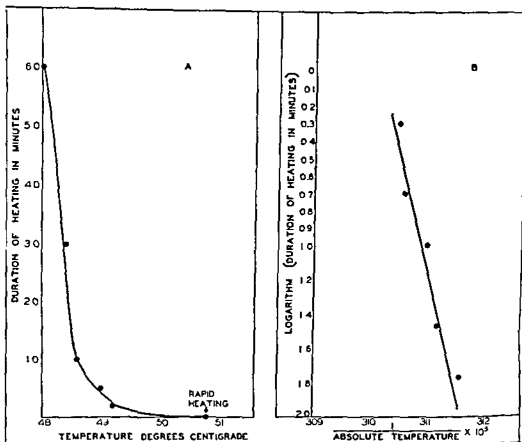


Fig 3 Relation Between Temperature and Duration of Heating of Normal Human Defibrinated Blood which Caused Approxi iately the Same Final Degree of Increased Osmotic Fragility

A (left) Duration of heating plotted against temperature B (right) The same data plotted as the logarithm of time (equivalent to log $\frac{1}{\text{rate}}$) against the reciprocal of the absolute temperature

The increase in o motic fragility that was selected as a basis of comparison was as follo s

H m l or	T nit y p d N Cl Gm c
1	o 74
2 5	o 68
5	o 55
10	o 43
50	o 34
75	o 30
95	o 4

The tonicities expressed here represent the average of several d terminations or extrapolations of data falling near the value given

C CHANGES PRODUCED BY HEAT AS A PROPERTY OF THE ERYTHROCYTE AND NOT OF THE MEDIUM

The changes in red blood cells produced by heat were investigated in order to determine whether the effects were reversible and whether inherent in the erythrocyte or dependent upon the presence of plasma or serum A sample of normal de

TABLE 3.—*Comparison of Morphologic Changes in Red Blood Cells in which Heating Produced a Similar Osmotic Fragility of Blood Samples Containing Normal Spherocytic or Hypochromic Erythrocytes*

Diagnosis	Temperature (of heating)	Hemolysis	Osmotic fragility of red blood cells: Hemolysis % 1	2.5	5	10	50	75	95	Morphologic characteristics of red blood cells: RBC	Hemoglobin	Hematocrit	MCV (Reticulocytes)	MCD Diameter (Morphology)	Standard Deviation From Mean Diameter	Coefficient of Variation of Diameters
			Tonicity as NaCl Gm %							millions/c mm	Gm %	%	micra³	micra	micra	%
Normal	Unheated		41	40	39	38	35	34	30	4.86	14.5	44.9	92 reticulocytes normal	7.3 (Normal morphology)	0.50	6.9
	50.6 C	0.6	82	79	76	72	39	34	25					4.7	1.6	35
Congenital hemolytic jaundice	Unheated		60	56	51	44	40	39	36	3.60	9.4	30.8	85 reticulocytes 11%	6.5 (spherocytes 3%)	0.74	11
	52 C	0.8	78	77	75	72	50	43	35					5.3	0.94	18
Sickle cell anemia	Unheated		48	37	33	29	22	17	08	4.78	9.8	32.3	66 reticulocytes 3%	6.8 (rare sickled forms target cells 12%)	0.96	14
	50.6 C	0.5	80	78	76	73	47	26	13					3.6 (rare sickled forms target cells 1%)	1.2	33

fibrinated human blood was kept at a temperature of 53 C for a period of nine minutes producing a marked increase in osmotic fragility The heated blood and a sample of unheated blood were then centrifuged and the serums removed and interchanged in such amounts as to produce a 5 per cent suspension of heated cells in unheated serum and the reverse These two mixtures together with unmanipulated samples of heated blood and unheated blood were then introduced into separate 250 cc tonometers which were closed and rotated slowly for two hours in an incubator at 37 5 C There was no change in the abnormally increased osmotic fragility of the heated red cells produced by the fresh unheated serum and no significant hemolysis or increase in the osmotic fragility of the unheated cells suspended in the

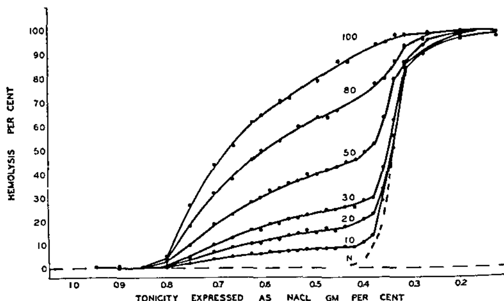


FIG 4 OSMOTIC FRAGILITY OF RED BLOOD CELLS IN MIXTURES OF HEATED AND UNHEATED NORMAL HUMAN DEFIBRINATED BLOOD

N unheated sample 100 (per cent by volume of blood) after rapid heating to 55 C 10–80 mixtures of varying proportions of the heated sample (10–80 per cent by volume) with the unheated sample

heated serum When mixtures of from 10 to 80 per cent by volume of heated blood exhibiting increased osmotic fragility were made with normal blood the resulting osmotic fragility curves showed the values to be expected from such a mixed population (fig 4)

In another experiment a sample of plasma (containing sodium citrate 500 mg per 100 cc) and of serum were heated at 56 C for two minutes and for thirty minutes respectively The precipitated fibrin was then removed from the heated plasma by centrifugation Five per cent suspensions of unheated red cells were then made in each and were incubated in tonometers as described above for the same time as unheated samples of whole citrated and defibrinated blood No significant changes occurred in the osmotic fragility or degree of hemolysis of any of the six samples

The effect of different mediums on the increases in osmotic fragility of red cells

caused by heat was investigated. Whole blood containing heparin 150 mg. per 100 cc., defibrinated blood, and red cells from defibrinated blood washed and resuspended in isotonic saline solution were heated for ten minutes in separate test tubes under identical conditions. At 48° C. a moderate and similar increase in osmotic fragility was produced in each. At 49.6 C., however, the red cells in isotonic saline showed a somewhat greater increase in fragility and a significantly greater degree of hemolysis, i.e., 16 per cent, compared to 2 and 1 per cent, respectively, for the other two samples. Similarly, washed red cells suspended in isotonic saline when heated up to 55 C. in three minutes showed a greater increase in osmotic fragility than did the red cells from defibrinated blood that was treated similarly. Hemolysis of the red cells in isotonic saline was 34 per cent but only 2 per cent in defibrinated blood. However, when the red cells of the heated defibrinated blood sample were washed and resuspended in isotonic saline, they exhibited the same increase in osmotic fragility as those originally heated in suspension in saline. In contrast to heparin, sodium citrate, 250 mg. per 100 cc., when used as an anticoagulant for whole blood or when added to defibrinated blood caused a significantly greater increase in fragility and in hemolysis when the samples were heated than was observed with heated defibrinated blood. The possible effect of other salts was not investigated.

The increase in osmotic fragility of the red cells was not accompanied by any significant change in the nonprotein nitrogen concentration of heated defibrinated blood or of erythrocytes washed and suspended in isotonic sodium chloride solution. There was no production of methemoglobin or sulphemoglobin. The hydrogen ion concentration of the suspension of red cells, as measured by the glass electrode, was not materially changed for heated whole blood containing heparin or sodium citrate 250 mg. per 100 cc., for defibrinated blood, or for erythrocytes washed and suspended in isotonic saline.

D. EQUILIBRIUM VOLUMES OF HEATED ERYTHROCYTES

The equilibrium volume of erythrocytes (hematocrit) from samples of normal and heated human defibrinated blood was tested by a modification of the method of Castle and Daland[11] who emphasize the fact that differences in osmotic fragility of different types of erythrocytes are not explained by differences in strictly osmotic properties but rather by differences in the shape of red cells. The purpose of these experiments was to determine what alterations, if any, were produced by heat in the permeability of the membrane of the red cell or in the osmotic activity of its contents. Because no striking changes in the hematocrit of samples of defibrinated blood were noted to occur as a result of heat, such effects appeared to be minimal.

Aliquot samples of human defibrinated blood were heated rapidly to 49.6 and 50.6 C., thus producing a slight and an extreme increase in osmotic fragility respectively, as shown in table 4. Then 1.0 cc. amounts of these two samples and of an unheated aliquot were mixed with 1.0 cc. amounts of solutions of sodium chloride ranging in concentration from 0.51 to 1.7 grams per cent. After mixing, the hematocrit of each sample was determined. Then the percentage difference between this value and the value obtained when the sample was mixed with sodium chloride solution 0.85 grams per cent was computed. The tonicities of the mixtures of serum

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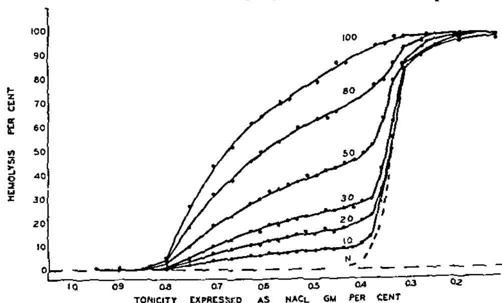


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In another experiment a sample of plasma (containing sodium citrate 500 mg per 100 cc) and of serum were heated at 56 C for two minutes and for thirty minutes respectively The precipitated fibrin was then removed from the heated plasma by centrifugation Five per cent suspensions of unheated red cells were then made in each and were incubated in tonometers as described above for the same time as unheated samples of whole citrated and defibrinated blood No significant changes occurred in the osmotic fragility or degree of hemolysis of any of the six samples

The effect of different mediums on the increases in osmotic fragility of red cells

heated samples of each were incubated in 250 cc tonometers with slow rotation at 37 5 C for periods of 4 8 or 10 hours A significant increase occurred in the os motic fragility of the heated compared to the unheated samples of dog blood The increases in the osmotic fragility of the heated human red cells were minimal even after ten hours as were those of the unheated controls This corresponds with pre vious observations[32] [33] upon the sterile incubation of normal and pathologic hu man blood and indicates apparently an increased susceptibility of the heated dog red cells to such incubation

F CHANGES IN MECHANICAL FRAGILITY OF ERYTHROCYTES PRODUCED BY HEAT

The mechanical fragility of samples of both heated and unheated human and dog blood was determined For example samples of defibrinated dog blood were sub jected to rapid heating at 49 8 52 8 and 54 C Immediately thereafter the hematocrit and osmotic fragility of each of the heated bloods were determined The hematocrits of the unheated samples were adjusted by removal of serum to equal those of the samples exposed to the highest temperature The hematocrits of the other heated samples were not adjusted in each experiment The mechanical fragility of a given sample was taken as the percentage of its hemoglobin liberated by standardized trauma from glass beads rolling in a rotating tonometer [20]

Heating of blood that was just insufficient to cause increase in osmotic fragility had no detectable effect on mechanical fragility However the effect of sufficient heat was to produce progressive increases in both the osmotic and mechanical fra gilities of the erythrocytes The increase in mechanical fragility was roughly pro portional to the increase in osmotic fragility Thus for example as may be seen from figure 5 rapid heating to temperatures of 52 8 and 54 C caused the mechani cal fragility of a sample of dog blood to increase from a control value of 7 0 per cent to 20 2 and 31 5 per cent respectively A temperature of 52 2 C caused the mechanical fragility of a sample of human blood to increase from a control value of 3 9 per cent to 16 3 per cent (no figure shown)

Continuous trauma of heated defibrinated human or dog blood for periods of from three to eight hours apparently did not destroy selectively those red cells that showed the greatest increase in osmotic fragility This was evidenced by observa tion of the curve of osmotic fragility at frequent intervals while hemolysis from trauma was progressing With human blood there was no evident change in shape of the osmotic fragility curve to indicate alteration of all or selective destruction of any particular portion of the cell population On the contrary samples of defibrin ated dog blood that were heated sufficiently to produce increased osmotic and me chanical fragility of the red cells when subjected to continuous trauma for fifty minutes or at intervals for three hours showed a significant uniform and progres sive increase in osmotic fragility as the hemolysis from trauma increased It was as sumed as a hypothesis without further investigation that the progressive increase in osmotic fragility resulting from the continuing trauma of heated dog blood might be the result of an increase in the degree of fragmentation of the red cells al ready initiated by heat

and salt solution were calculated, assuming the serum tonicity to be isotonic with 0.85 grams per cent sodium chloride solution. In the experiment shown in table 4 the original red cell volume was 48, the serum volume 52 per cent. The hematocrit readings were probably only approximate in accuracy because of the polymorphic nature of the heated red cells. Moreover, because of their increased osmotic fragility, a significant portion of the heated red blood cells hemolyzed in the hypotonic mixtures of sodium chloride. *In the hypertonic solutions, however, hemolysis did*

TABLE 4.—*Change in Equilibrium Volume of Unheated and Heated Normal Defibrinated Human Blood Mixed With an Equal Volume of Hypotonic or Hypertonic Solutions of Sodium Chloride and Compared to the Hematocrit in Isotonic Sodium Chloride*

Concentration of solution of NaCl Gm % mixed with blood	Calculated tonicity of mixture of serum and NaCl solution expressed as NaCl Gm %*	Control unheated		Rapid Heating to 49.6 C		Rapid Heating to 50.6 C	
		Hematocrit	Change in hematocrit	Hematocrit	Change in hematocrit	Hematocrit	Change in hematocrit
		%	%	%	%	%	%
1.7	1.41	19.0	−20.1	19.0	−19.5	19.1	−20.4
1.36	1.19	20.2	−15.8	20.1	−14.8	21.0	−12.5
1.19	1.08	21.0	−12.5	21.7	−8.0	21.7	−9.6
1.02	0.96	22.0	−8.3	22.3	−5.5	22.9	−4.6
0.85 (isotonic)	0.85	24.0	0	23.6	0	24.0	0
0.68	0.74	25.1	+4.6	25.6 Hemolysis ±	+6.8	Hemolysis 2+	—
0.51	0.63	27.2	+13.3	Hemolysis +	—	Hemolysis 3+	—
		Tonicity as NaCl Gm %					
Osmotic fragility Hemolysis %	1	41		79		81	
	5	39		62		77	
	10	37		44		76	
	50	33		34		59	
	75	32		26		39	

* Serum was considered to have a tonicity equivalent to NaCl 0.85 Gm. per cent.

not occur and the percentage decrease in hematocrit was roughly the same for the heated and unheated sample. This indicates that, as with red cells of naturally occurring different osmotic fragilities, the strictly osmotic behavior (percentage change in equilibrium volume with change in tonicity of suspension medium) of the heated red cells did not differ significantly from that of the normal red cells.

E. EFFECT ON HEATED ERYTHROCYTES OF SUBSEQUENT INCUBATION AT 37.5 C

Samples of normal human and dog defibrinated blood were so heated as to produce a moderate increase in osmotic fragility. Then 6 cc. amounts of heated and un

and 8 the mechanical fragility of the red cells was also determined All urine samples were collected The rectal temperature was recorded at frequent intervals

Dog 1 received his own red cells which were heated in serum and then washed and resuspended in saline as described below in order to eliminate any potential hemolytic or toxic factor contained in the heated serum From Dog 1 weighing 12 Kg approximately ⅓ of the calculated blood volume 250 cc was withdrawn defibrinated heated in a water bath at 54 C during a period of twelve and one half minutes required for the temperature of the blood to reach 52 2 C The sample was then immediately cooled to body temperature centrifuged the serum discarded and the red cells washed three times with 2 volumes of hypertonic (1 2 5 grams

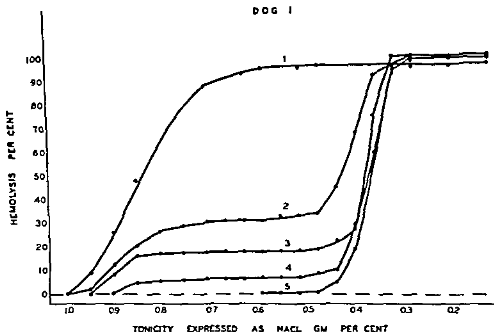


FIG 6 DOG 1 EFFECT OF INTRAVENOUS INJECTION OF WASHED RED BLOOD CELLS FROM 250 CC HEATED DEFIBRINATED DOG S BLOOD UPON OSMOTIC FRAGILITY OF CIRCULATING RED BLOOD CELLS

Curve 1 Washed red cells before intravenous injection Curve 2 immediately after injection Curve 3 after 2 hours Curve 4 after 7½ hours Curve 5 after 41 hours See also figure 7 Note that approximately 30 per cent of the red cell population was abnormal immediately after injection (curve 2) Subsequently the osmotically fragile red cells progressively disappeared

per cent) sodium chloride solution After the red cells were resuspended at their original hematocrit in the hypertonic saline they exhibited an extreme increase in osmotic fragility as shown in curve 1 in figure 6 Immediately after the intravenous injection of the suspension the animal s venous blood showed a mixed red blood cell population with approximately 30 per cent of cells of markedly increased osmotic fragility and o per cent of normal cells as indicated in curve 2 in figure 6 This type of osmotic fragility curve resembled that occurring in artificial mixtures of heated and unheated blood as illustrated in figure 4 The subsequent osmotic fragility curves showed gradual disappearance of the abnormally fragile cells during eighteen hours after injection Immediately after the injection of the heated blood although no free hemoglobin was detectable in the suspension of washed red cells injected a large amount of hemoglobin was noted in the animal s plasma and later hemoglobinuria appeared as shown in figure 7 The maximum value for plasma hemoglobin was 1 67 grams per 100 cc that for urine hemoglobin was

Effect of Injection of Heated Erythrocytes into Dogs

In previous observations[1] on patients with thermal burns hemoglobinemia and hemoglobinuria were found It is clear from the preceding experiments that in blood samples heated in vitro striking increases in both osmotic and mechanical fragilities of the red cells were demonstrated In order to determine whether such red cells would be readily destroyed in vivo blood was removed from normal dogs heated and injected into the same animal

In the several experiments 8 healthy dogs in the fasting state weighing from 12 to 18 kg were bled using sterile precautions, from the jugular or femoral veins

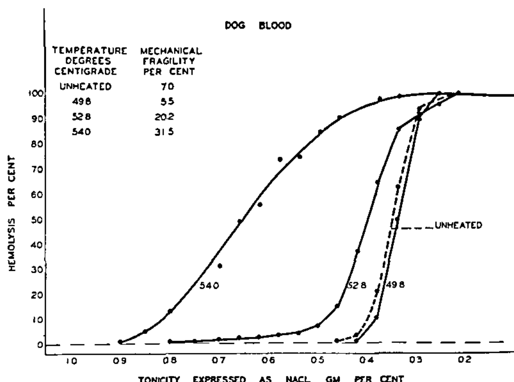


Fig 5 Osmotic and Mechanical Fragilities of Red Blood Cells of Dog's Defibrinated Blood after Rapid Heating to 49 8 52 8 and 54 0 C Respectively as Indicated

from $\frac{1}{6}$ to $\frac{1}{3}$ of their blood volume as estimated from their body weight [31] The blood was defibrinated or citrated as drawn In Dogs 1 and 5 ether anesthesia with an open cone was used briefly during the venesection Immediately after the bleed ing an equal volume of isotonic sodium chloride solution containing 40 cc of molar sodium lactate was injected intravenously The blood sample removed was treated as described below and later injected intravenously into the same animal Thereafter the animal was given food and water *ad libitum* Samples of venous blood were taken at frequent intervals before and after the injection with pre cautions to prevent hemolysis of the samples by trauma [4] On these samples were determined especially the morphology and osmotic fragility of the erythro cytes the plasma hemoglobin [4] and the hematocrit In experiments on Dogs 6 7

per cent of the red cells Immediately after injection of this suspension the animal s venous blood showed a mixed population containing approximately 15 per cent of red cells of extremely increased osmotic fragility which disappeared progressively within seven hours as shown in figure 8 Stained smears showed 8 per cent of spherocytes immediately after injection without evidence of the numerous small red cell fragments observed in the blood after it was heated in vitro Apparently these fragments were

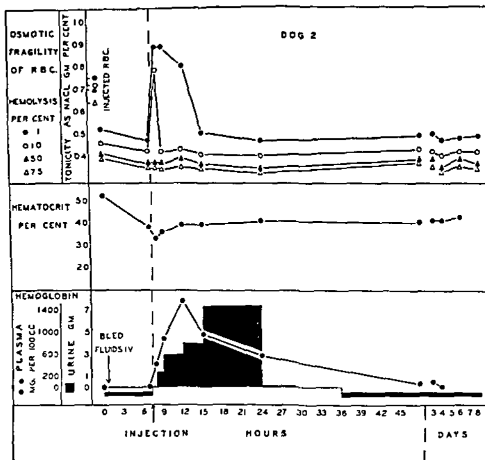


FIG 8 DOG 2 EFFECT OF INTRAVENOUS INJECTION OF WASHED HEATED RED BLOOD CELLS FROM 340 CC OF CITRATED DOG 6 BLOOD

As shown in the upper left hand corner of figure the injected red cells exhibited marked increase in osmotic fragility Note the prompt and progressive hemoglobinemia and hemoglobinuria accompanied by the rapid removal of the osmotically fragile cells

rapidly removed in the animal leaving spheroid erythrocytes which disappeared progressively from the blood stream coincident with the decreasing osmotic fragility The maximum concentration of hemoglobin in the plasma was 1 65 grams per 100 cc the maximum in the urine was 2 10 grams per cent Of approximately 55 grams injected a total of 6 4 grams of hemoglobin was recovered in the urine

Dog 3 ... ed he ted whole defibrinat d blood From Dog 3 weighing 12 Kg approximately ½ of the calculated blood volume 160 cc was removed defibrinated and heated in a water bath at 54 2 C for two periods of heating first to a temperature of 52 8 C requiring ten minutes and second to a temperature of 53 2 C requiring four and one half minutes This heating produced a large increase in osmotic

2 0 grams per cent Only 6 4 grams of hemoglobin were recovered in the urine from approximately 40 grams of hemoglobin that was injected There was no significant loss in the collection of urine specimens

Dog 2 received his own red cells which were first washed and resuspended in saline and then heated in order to eliminate any effects of the plasma upon the red cells during the process of heating From Dog 2 weighing 18 kg approximately ⅓ of the calculated blood volume 340 cc was withdrawn into 20 cc of a solution of 11

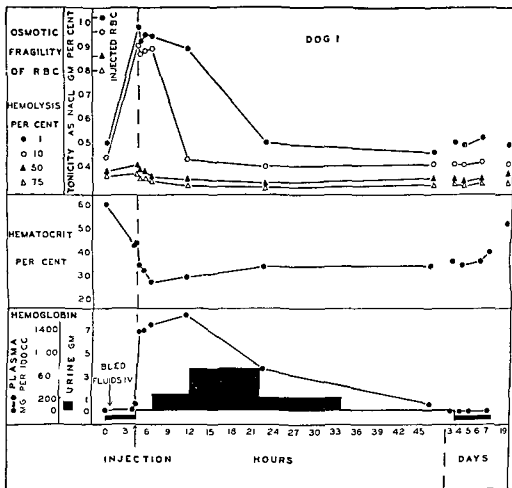


Fig 7 Dog 1 Effect of Intravenous Injection of Washed Red Blood Cells from 250 cc Heated Defibrinated Dog's Blood

The injected red cells exhibited the marked increase in osmotic fragility shown in the upper left hand corner of the figure Note the immediate maximal hemoglobinemia and subsequent hemoglobinuria *despite the absence of free hemoglobin in the red cell suspension injected and the rapid disappearance* of the osmotically fragile cells

grams per cent sodium citrate making a final concentration of 0 6 grams of sodium citrate per 100 cc of blood The sample was centrifuged the plasma discarded and the red cells washed three times with three times their volume of isotonic 0 85 grams per cent sodium chloride solution The red cells re suspended at their original hematocrit in isotonic saline were then heated in a 500 cc Erlenmeyer flask with gentle agitation in a water bath at 55 C for two periods of heating first to a temperature of 52 5 requiring seven and one half minutes and second to a temperature of 52 7 C requiring seven and one fourth minutes This heating produced a large increase in osmotic fragility and caused hemolysis of 7

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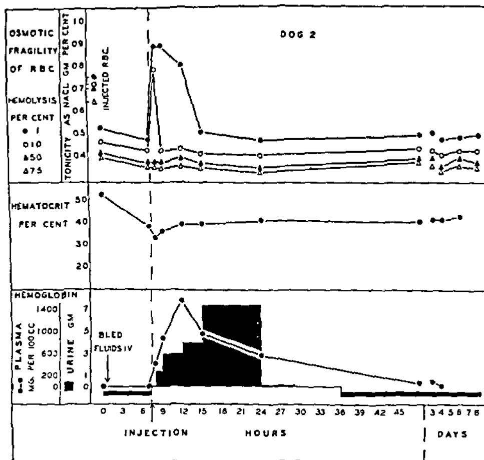


Fig 8 Dog 2 Effect of Intravenous Injection of Washed Heated Red Blood Cells from 340 cc of Citrated Dog s Blood

As shown in the upper left hand corner of figure the injected red cells exhibited marked increase in osmotic fragility Note the prompt and progres ive hemoglobinemia and hemoglobinuria accompanied by the rapid removal of the osmot cally f agile cells

rapidly removed in the animal leaving spheroid erythrocytes which disappeared progressively from the blood stream coincident with the decreasing osmotic fragility The maximum concentration of hemoglobin in the plasma was 1 65 grams per 100 cc the max mum in the urine was 2 10 grams per cent Of approximately 55 grams injected a total of 16.4 grams of hemoglobin was recovered in the urine

D g 3 r ited h ated wh le d fib nat b l From Dog 3 weighing 12 kg approxim tely ⅓ of the calculated blood vol me 160 cc vas r moved defibrinated and heated in a water bath at 54 2 C for two periods of heating first to a temperature of 52 8 C requi ng ten minutes and second to a temperature of 53 2 C requiring four and one half m n tes This heating produced a large increase in osmoti

fragility and 7 per cent of the red cells were hemolyzed Immediately after injection approximately 10 per cent of the animal s red cells showed a marked increase in osmotic fragility and 8 6 per cent of the cells in the stained smear were identified as spherocytes No red cell filaments were seen The proportion of cells showing increased fragility and spheroidicity decreased to 5 per cent one half hour after injection and to approximately 1 per cent two hours after injection As shown in figure 9 the maximum

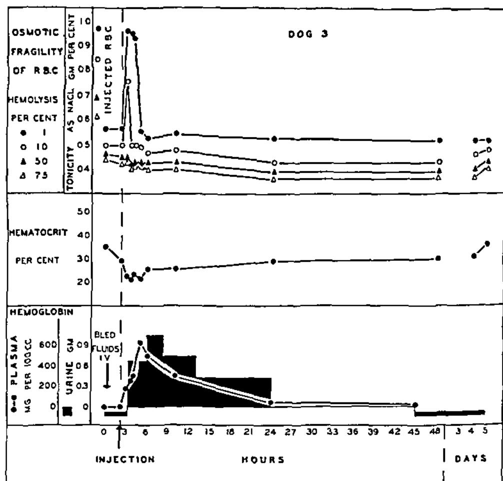


Fig 9 Dog 3 Effect of Intravenous Injection of 160 cc of Heated Defibrinated Dog s Blood

As shown in the upper left hand corner of the figure the injected red cells exhibited marked increase in osmotic fragility Note the prompt and progressive hemoglobinemia and hemoglobinuria accompanied by rapid removal of the osmotically fragile cells

concentration of hemoglobin in the plasma was 610 mg per 100 cc three hours after the injection the maximum in the urine was 240 mg per 100 cc Of approximately 20 grams injected a total of 3 1 grams of hemoglobin was recovered in the urine

These three animals Dogs 1 2 and 3 showed a copious diuresis of from 800 to 700 cc of urine during the period of hemoglobinuria which lasted from thirty to forty eight hours after injection The urine pH varied from 6 5 to 8 3 but was usually alkaline The animals developed no general reaction and no azotemia One animal only Dog 2 showed a rise in temperature (3 degrees F) during a period of from one to seven hours after the injection The animals showed an increase in leucocyte count which reached a maximum of from 27 600 to 39 000 in from twenty one to forty two hours after injection of

the heated blood samples A moderate increase in the number of reticulated red cells of from 2 to 9 per cent occurred in from four to six days after injection of the heated blood

As a control experiment Dog 4 r eived blood heated insuffici ntly to ca se increase in osmotic f agility From Dog 4 weighing 12 kg approximately ⅓ of the calculated blood volume 250 cc was removed de fibrinated heated in a liter Erlenmeyer flask with agitation in a water bath at 50 5 C for a period of nine minutes when the temperature of the blood reached 50 C It was then cooled to room temperature This heating caused no change in osmotic fragility The intravenous injection of the blood caused as

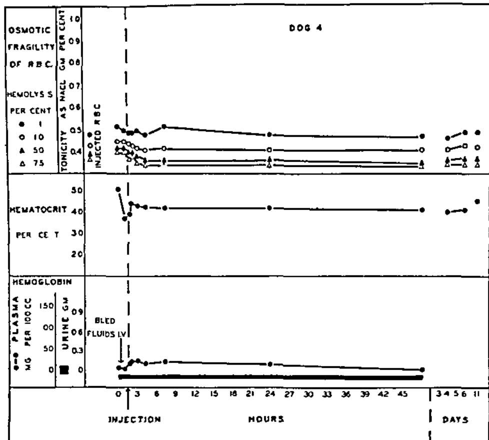


Fig 10 Dog 4 Negative Effect upon Osmotic Fragility o Circulating Red Blood Cells of Intravenous Inj ct on of 250 cc of Defibrinated Dog s Blood Heated Just Insufficiently to Cause Changes in Osmotic Fr gility

sho n in figure 10 no change in the osmotic fragility of the erythrocytes in the animal s blood stream no apparent destruction of red blood cells and no hemoglobinemia or hemoglobinuria

In another co trol exp m nt Dog 5 rece ed only plasma obta ned f m he ted bl d sh u g increased osm tic f agility of the erythrocytes From Dog 5 weighing 15 kg approximately ⅓ of the calculated blood volume 300 cc was removed into sufficient 11 per cent sodium citrate solution to make a fin l concentration of 0.4 grams per 100 cc of blood This sample was heated in a 500 cc Erlenmeyer flask with gentle agitation in a water bath at 54 5 then 55 C for two periods of heating first to a temperature of 53 3 C for the blood requiring eight minutes and second to a temperature of 53 8 C requiring six minutes A m ximum incre se in the osmotic fragility and hemolysis of 7 per cent of the red cells was produced the heated blood was then cooled centrifuged for one hour at 3000 r p m the plasma remo ed and

the red blood cells and precipitated fibrin discarded The plasma 120 cc was red contained 725 mg of hemoglobin per 100 cc and was opaque due to its content of masses of particulate matter of approximately the size of bacteria These particles did not appear to be fibrin which was removed in the sediment but probably were filaments of red blood cells which were always seen after heating defibrinated blood or red cells suspended in serum or isotonic salt solution Following intravenous injection of the plasma as shown in figure 11 there was no change in the osmotic fragility of the dog s red cells and no decrease

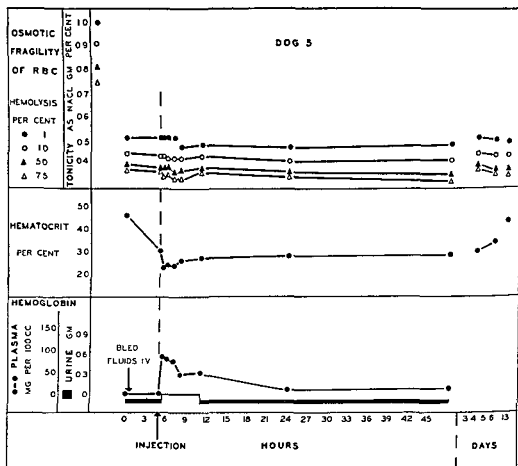


Fig 11 Dog 5 Negative Effect upon Osmotic Fragility of Circulating Red Blood Cells of Intravenous Injection of Plasma from 300 cc of Heated Citrated Dog s Blood

As indicated in the upper left hand corner of the figure the discarded red blood cells showed a marked increase in osmotic fragility The moderate immediate rise in plasma hemoglobin is due entirely to the plasma injected which contained 725 mg of hemoglobin per 100 cc

in the hematocrit subsequent to that produced immediately by discarding approximately ⅓ of the animal s erythrocytes The maximum level of hemoglobin in the plasma which was obtained immediately after the injection was 84 mg per 100 cc and can be a counted for entirely as derived from the hemoglobin contained in the injected plasma There was copious excretion of urine containing only a trace of hemo globin following the injection of the approximately 8 0 mg of hemoglobin contained in the heated plasma

At this point in the investigation the mechanical fragility of the heated red blood cells was recognized as a possible important mechanism to account for their prompt destruction in vivo Accordingly in

Dogs 6 7 and 8 observations were made of both the osmotic and mechanical fragilities of the blood heated in vitro and of blood samples obtained from the dog after the injection of the heated blood

Dog 6 re tired heat d whole defibrinated blood From Dog 6 weighing 14 Kg approximately ⅓ of the calculated blood volume 335 cc was withdrawn and defibrinated Of this sample 227 cc or about ⅕ of the animal s blood volume was heated in a water bath at 55 5 C requiring six and two thirds minutes to reach a temperature of 53 C Determination of the osmotic fragility of the heated blood showed significantly increased values as follo s 1 per cent hemolysis in 0 90 10 per cent in 0 83 50 per cent in 0 44 and 75 per cent in 0 38 grams per cent solution of sodium chloride The mechanical fragility of the heated sample was 26 per cent compared to 3 2 per cent for an unheated control portion Immediately after injection of the heated blood approximately 20 per cent of the animal s red cells showed increase in osmotic fragility and 10 per cent of cells were identified as spherocytes a value which decreased to 1 7 per cent at the end of twenty four hours The mechanical fragility of the animal s blood was 26 9 per cent One half hour after the injection the mechanical fragility was elevated to the maximum figure of 65 per cent and thereafter gradually decreased to normal by the end of forty-eight hours The hemoglobin in the plasma was greatest three hours after the injection 685 mg per 100 cc the maximum concentration in the urine was 248 mg per 100 cc Of approximately 30 Gm injected a total of 0 58 Gm of hemoglobin was recovered in the urine No figure is shown for this experiment

Because of the unexplained extreme elevation of the mechanical fragility of the blood of Dog 6 after injection of the heated defibrinated blood the experiment was repeated

Dog 7 rece ed heat d whole defib nated blood From Dog 7 weighing 16 8 Kg approximately ⅓ of the calculated blood volume 345 cc was withdrawn and defibrinated Of this sample 265 cc was heated in a water bath at 56 5 to a temperature of 53 5 C which required a period of four and one third minutes This heating produced a large increase in osmotic fragility as follows 1 per cent hemolysis in 0 95 10 per cent in 0 89 50 in 0 82 and 75 in 0 75 grams per cent solution of sodium chloride During heating 4 2 per cent of the red cells were hemolyzed The mechanical fragility of the sample was 31 per cent compared to 3 9 per cent for an unheated portion Immediately after injection of 245 cc of the heated blood amounting to about 15 per cent of the animal s blood volume approximately 12 per cent of the red blood cells showed extreme increase in osmotic fragility Six and six tenths per cent of the cells were identified as spherocytes a value which decreased to 0 4 per cent by the end of four hours The mechanical fragility of the animal s blood which was 7 1 per cent immediately after injection reached a maximum figure of 20 per cent at the end of four hours and declined to normal by twenty four hours The maximum concentration of hemoglobin in the plasma 203 mg per 100 cc occurred one and one half hours after the injection the maximum in the urine was 254 mg per 100 cc Hemoglobinuria was observed for a period of twenty four hours but the total amount excreted is not available No figure is shown for this experiment

In *Dog 8* three sets of observations were made on three consecutive days

On Day 1 the animal weighing 9 6 Kg was bled about ⅓ of the calculated blood volume 425 cc which was at once replaced by the intravenous injection of an equal volume of physiologic salt solution The blood sample was defibrinated centrif ged the serum removed the red cells suspended in 50 cc of physiologic salt solution and both the serum and red cells stored in the ice box The venesection and injection of the saline produced no change in osmotic fragility no hemoglobinemia or hemoglobinuria Likewise as shown in figure 12 there was no change in the mechanical frag lity of the animal s blood

On Day 2 130 cc of serum from the animal as heated in a water bath at 56 4 C to reach a temperature of 53 5 C after two and three fourths minutes Following injection of the serum there was no change in osmotic fragility no hemoglobinemia no hemoglobinuria and as shown in figure 12 no change in the mechanical fragility of the animal s circulating red cells During Days 1 and 2 the decrease in the level of the hematocrit of the animal s blood was consistent with the amount of red cells removed and the serum that was replaced

On Day 3 270 cc of the packed red cells drawn on Day 1 were suspended in physiologi saline and heated in a water bath at 55 6 C, to reach a temperature of 52 C after ten and one sixth minutes This heating produced the marked increase in the osmotic fragility of the red cells shown in the upper right hand corner of figure 12 It c sed an elevation of the mechan cal fragility of the red cell suspension to 17 3 per cent Immediately after the injection of the heated suspension of red cells approximately 25

per cent of the circulating red cells exhibited a striking increase in osmotic fragility Eight per cent of the cells were identified as spherocytes a value which decreased to 0 8 per cent twenty four hours after the injection As shown in figure 12 the mechanical fragility of the blood sample withdrawn immediately

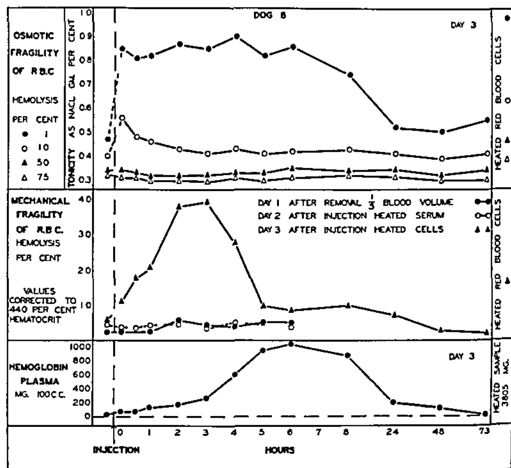


Fig 12 Dog 8 Comparative Effects of Removal of Blood and Injection of Physiologic Salt Solution (Day 1) Intravenous Injection of Heated Dog Serum (Day 2) and Intravenous Injection of Heated Washed Red Blood Cells (Day 3)

Day 1 Note negative effective on mechanical fragility of removal of 425 cc of blood by venesection and injection of an equal volume of physiologic salt solution

Day 2 Note negative effect on mechanical fragility of injection of 130 cc of heated serum

Day 3 Note increases in osmotic and mechanical fragilities of red cells and progressive early hemo globinemia resulting from intravenous injection of 270 cc of heated red cells suspended in physiologic saline The osmotic fragility of the heated red cells before injection is shown in the upper right hand corner of the chart

after the injection was 11 8 per cent and this increased to 38 and 39 per cent two to three hours respectively later Thereafter the mechanical fragility fell rapidly during the next two hours and returned to normal by forty eight hours after the injection The maximum concentration of hemoglobin in the plasma 1 0 Gm per cent occurred at the end of five hours There was manifest hemoglobinuria during Day 3 which disappeared twenty four hours after the injection

CHARACTERISTICS OF THE RED CELLS IN A CASE OF FATAL THERMAL BURNS

Observations have already been reported[1] on 14 patients with moderate or severe thermal burns Similar observations are reported by Brown [14, 15] In the present study the characteristics of the red blood cells were investigated with particular

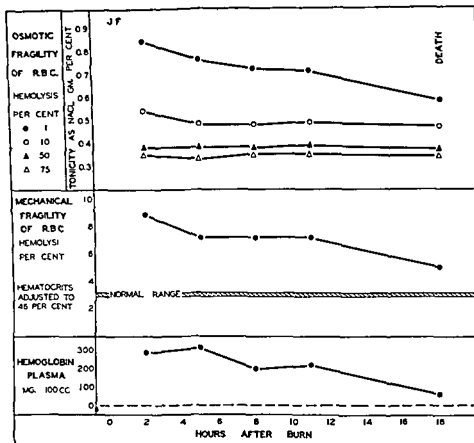


FIG 13 CASE 15 OSMOTIC AND MECHANICAL FRAGILITIES OF RED BLOOD CELLS AND PLASMA HEMOGLOBIN IN A PATIENT WITH A FATAL THERMAL BURN

Note the initial elevation and the subsequent simultaneous and progressive decline towards normal of these elevated values

reference to their mechanical fragility in an additional instance of fatal thermal burns The patient Case 15 in the series* was a 45 year old male who was admitted to the Boston City Hospital one half hour after receiving second and third degree burns of approximately 75 per cent of the skin area He received prompt and continuing treatment including the administration of 3950 cc of pooled plasma and 1050 cc of pooled serum during the sixteen hours before his death The patient showed moderate hemoconcentration hemoglobinemia and hemoglobinuria but no azotemia The results of the examinations of the blood are shown in figure 13

* Cases 1–14 were reported previously [1]

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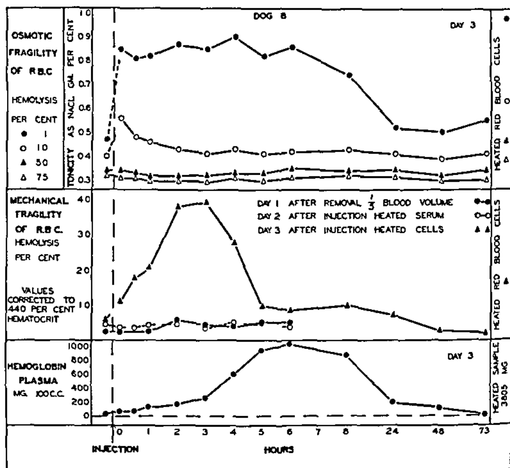


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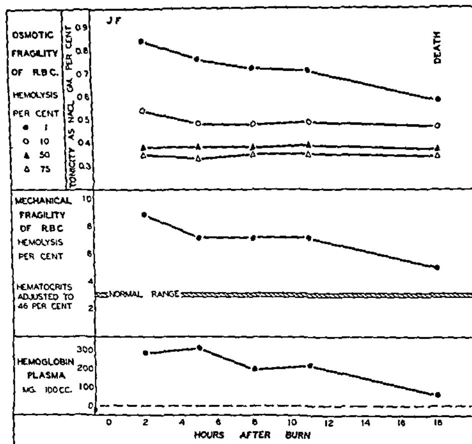


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Two hours after the burn, the osmotic fragility of approximately 20 per cent of the red cells was markedly increased Ten per cent of the red cells were identified as spherocytes a value which persisted for 18 hours The mechanical fragility of the red cells initially was increased to 8 5 per cent and had decreased by the end of 18 hours to approximately half of its original value The maximum concentration of hemoglobin in the plasma was observed to be 306 mg per 100 cc about five hours after the burn

Discussion

It has been demonstrated here and elsewhere[1 7 8] that blood heated under the conditions described above shows progressive division of the erythrocytes with the formation of numerous spheroid cells of various sizes together with an increase in osmotic fragility The present experiments demonstrate an increase in the mechanical fragility of the red cells and the remarkable fact that subdivision may be accomplished without loss of cell contents i e without hemolysis The mechanism by which heat causes this fragmentation is unknown However two aspects of the problem are considered here first the relation of repeated red blood cell division to increased osmotic and mechanical fragilities and second the mechanism of destruction of such red blood cells in vivo

With regard to the nature of the cell division induced by heat it was repeatedly noted that under appropriate conditions considerable division of the red blood cells could occur in plasma serum or isotonic salt solution without the liberation of any appreciable amount of hemoglobin As most modern observers consider the erythrocyte to consist of a distinct membrane surrounding a liquid interior its division without loss of hemoglobin implies that this process was accomplished without loss of continuity of the envelope of either parent or daughter form even at the point of their separation That the cell contents were not altered by heating in such a way as to have prevented their escape in the presence of a defect in the cell envelope is shown by the fact that hemoglobin left the cells whenever with sufficient heating any of them became so fragile as to be ruptured by isotonic salt solution or when heated blood was mechanically traumatized Moreover in distilled water total hemolysis occurred as usual

Despite the striking morphologic phenomena of cell division the osmotic activity of the cell contents was not materially changed by heat This was shown by the fact that the process of heating blood caused only a slight apparent increase in the red cell volume (hematocrit) in plasma possibly due in large part to difficulty in packing the new irregular forms in the centrifuge This observation also indicates that the permeability of the membrane of the heated red cells was not significantly altered with respect to plasma electrolytes That it remained relatively normal for sodium chloride and water was demonstrated by the parallel reductions in equilibrium volumes (hematocrit) of normal and heated red cells when suspended in hypertonic solutions of sodium chloride As already reported[3] for normal blood heated dog red cells exhibited progressive increases in volume and in osmotic fragility on sterile incubation at 37 C These facts indicate that the parent and

daughter cells produced by division do not behave osmotically in a significantly different fashion from normal red cells at least under the conditions of the present experiments It should be mentioned in passing that the erythrocyte serves as a convenient biologic form for the quantitative evaluation of thermal injury

Thus from the evidence available it appears that the process of red cell division caused by heat results largely in morphologic changes tending on the average toward an increase in spheroidicity The causal relation of increased spheroidicity to increased osmotic fragility of the red cells of certain animal species and pathologic conditions in man has already been pointed out [11, 15–17] Castle and Daland[11] showed that red cells of widely differing osmotic fragilities exhibited entirely similar percentage increases in equilibrium volumes when suspended in plasma diluted with water to various tonicities Consequently they were able to infer from calculation of the cell surface area that for each type of red cell the percentage difference between the discoidal volume in isotonic solution and that of a sphere with a surface area equal to that of the discoid form provides a measure of its osmotic fragility The osmotic fragility of a red cell is thus an index of the amount of swelling that can occur in hypotonic solutions before the discoid form becomes spherical The sphere is the critical form as it contains the greatest volume within the least surface A further increase in volume inevitably involves increase in surface Experimentally this results in the escape of the contents of the red cell including the hemoglobin presumably as a result of rupture of the plastic but relatively inelastic cell membrane

The effect of heat on red cells was patently to cause division and it was also noted that many of the divided forms appeared to be spherocytes of various sizes Recollection that the evidence cited above indicates that the strictly osmotic characteristics (not the osmotic fragility) of the red cells did not appear to be significantly modified by heat invites a theoretic consideration of the geometric implications of multiple subdivision of a discoid object with a membrane enclosing liquid contents *without increase in either the volume or the surface area and without loss of cell contents* Obviously division of an object does not increase the combined volumes of the new forms produced However the first division will result in an increase of their combined surface area Since increase in surface is either entirely contrary to or becomes a limiting factor in the apparent experimental conditions an economy of the surface to volume ratio must be effected The shape having the greatest economy of surface for its volume is the sphere *Consequently each cell division must involve a progressive approach to the spherical shape for one or both new elements* If as appeared under the microscope some small elements are formed consisting largely of surface the parent object must have been even more effectively deprived of surface compared to volume After the spherical form is reached a further division will be impossible without stretching of the cell membrane In this circumstance escape of cell contents including hemoglobin is inevitable The average effect of red cell division by heat is thus to produce spheroid cells which for the reasons given either rupture in the process of further division or are easily ruptured in hypotonic solutions This provides an adequate explanation of the greater

effectiveness of heat in causing an increase in osmotic fragility (approach to spherical form) of the initially more nearly spherical cells of congenital hemolytic jaundice

For the geometric reasons just discussed it is believed that heated red blood cells may also exhibit increased fragility to mechanical trauma under standardized conditions It cannot be denied however that a part of or even the major cause of the increase in mechanical fragility of the heated red cells is a decrease in the inherent strength of the cell envelope The coincidence of increased osmotic and mechanical fragilities of red cells which has been observed after heating and as a result of certain other experimental conditions reported elsewhere [0] nevertheless suggests the possibility of a common basis for both namely the spheroid shape of the erythrocyte A red cell may be so nearly spherical that a slight increase in volume induced by osmotic means will rupture the cell membrane If this spheroid red cell received the impact of a glass bead it will become momentarily less nearly spherical This change however will require according to the laws of geometry more surface to cover the same volume and may result in rupture of the cell membrane A risk to the continued integrity of the surface of the red cell from mechanical trauma is thus seen to be created by the same critical surface–volume ratio for the red cell that determines its osmotic fragility Correspondingly fewer nearly spherical cells will successfully withstand the deformity of trauma Indeed the considerable deformity imposed upon red blood cells in traversing small capillaries makes the biologic value of the discoid form clearly apparent

The second problem for discussion is the mechanism of the destruction of the red blood cells in patients with thermal burns It was demonstrated that heating dog s blood insufficiently (50 C) to cause change in osmotic fragility caused no change in mechanical fragility and did not produce increased blood destruction when these erythrocytes were injected into the dog Similar observations are reported[17] for animals heated to a temperature of 47 C in hot water Also plasma obtained from heated dog s blood showing a large increase in the osmotic fragility of the erythrocytes caused no hemolysis or change in the osmotic or mechanical fragilities of the circulating red blood cells when the plasma was injected intravenously When however by sufficient heating (52 2 to 53 2 C) some of the red blood cell population became so osmotically fragile as to hemolyze in isotonic saline the mechanical fragility of the red blood cells was found to be from 17 to 31 per cent compared to from 3 to 5 per cent for the unheated blood Similar results were obtained whether the red cells were washed in saline before or after heating or whether heated and injected in serum plasma or saline

Immediately after the injection into dogs of such erythrocytes the venous blood contained spherocytes and exhibited an increased osmotic fragility curve the form of which depended entirely upon the proportion of heated cells mixed with the normal red blood cells of the animal Likewise the mechanical fragility of the circulating blood immediately after the injection of the heated red blood cells showed an increase that corresponded to the proportion of mechanically fragile cells (7 to 26 per cent) The subsequent remarkable increase of the mechanical fragility without further increase of the osmotic fragility of the red cells was sometimes of such mag

nitude (e g 65 per cent in Dog 6) as to predicate an effect on the unheated red cells as well This finding has not been explained but was shown not to be due to change in the properties of the plasma (autoagglutinins) and did not appear after heated blood was incubated in vitro Also no increase in osmotic or mechanical fragility resulted from the injection into the dog of large amounts of stroma prepared from dog red blood cells Because the unpredicted increase in mechanical fragility was unaccompanied by any increase in osmotic fragility it is probably although consistently found due to some property of the artificial in vitro conditions of the test for mechanical fragility

Hemoglobinemia soon became maximal and later hemoglobinuria was marked and indicated rapid destruction of many of the circulating red blood cells The spherocytes serving as identifiable heated cells, and cells with increased osmotic fragility disappeared progressively and were completely absent by the end of from four to twenty four hours In the three experiments in which measurements were made the mechanically fragile red cells also disappeared in from twenty four to forty-eight hours The hematocrit of the circulating dog s blood did not decrease below the level apparently resulting from the removal of the blood samples and hemolysis of the heated red blood cells From these data it appeared probable that the heated red cells which were spheroid and exhibited both increased osmotic mechanical fragilities were selectively destroyed

In the patient with fatal thermal burns, Case 15 spherocytes were observed in the circulation and the osmotic and mechanical fragilities of the red cells were maximal at first and later declined towards normal as in the dogs injected with heated red cells Similarly in Cases 4 and 14 of the series of patients with burns previously reported[1] and as reported by Brown [14 15] the osmotic fragility was initially at its highest value and subsequently as in the dog experiments decreased progressively for eighteen hours or longer if the patient survived Although all these patients received plasma or plasma and serum in large amounts they did not show the progressive increase in osmotic fragility reported by Ebert and Emerson[38] in patients of blood groups A or B who received pooled plasma or whole blood of Group O It is therefore believed that the effect of incompatible blood plasma upon the osmotic fragility of the erythrocytes is not operative in the cases of burns reported in this study

It seems obvious that those red blood cells susceptible to hemolysis in isotonic salt solution would also rupture in vivo in isotonic plasma For those red blood cells however *showing increased susceptibility to hemolysis only in hypotonic salt solutions* the mechanism of destruction in the isotonic conditions of the body required discussion It has already been demonstrated [0 3 33] that sterile incubation at 37 5 C of normal human blood in vitro with limited access to fresh serum and probably intravascular stagnation of animal blood in vivo lead to progressive increases in volume spheroidicity osmotic and mechanical fragility of the red cells such that eventually some of them hemolyze in isotonic salt solution plasma or serum The red cells of congenital hemolytic jaundice are especially susceptible to this process both in vitro and when sequestered in the spleen [39 40] Likewise in observations on heated dog blood it was demonstrated that red cells which exhibited

increased osmotic fragility showed further increase in osmotic fragility on incubation in vitro Therefore it is probable that in the injection experiments the already osmotically fragile heated red cells would like those of congenital hemolytic jaundice be especially susceptible to destruction by intravascular stagnation in such locations as the spleen and liver

The rate of destruction of the heated red cells following intravenous injection into the dogs was, however manifestly more rapid than occurred on incubation of the sterile blood in vitro Thus whereas in the dog most of the heated red cells disappeared from the circulation in one instance by the end of four hours and always by the end of twenty four hours in the test tube little if any increase in osmotic fragility was evident after four hours and the definite increase in osmotic fragility that appeared in twenty four hours was accompanied by only 15 to 30 per cent hemolysis Therefore a more likely explanation of their rapid rate of destruction in vivo appears to be the increased mechanical fragility which invariably accompanied the increased osmotic fragility of the heated red cells Consistent with this explanation is the attainment of maximal plasma hemoglobin values within the first six hours and the complete disappearance of the mechanically fragile cells within twenty four hours following the injection In the fatally burned patient Case 15 the time relations of the disappearance of the mechanically fragile red cells also fit this hypothesis

The reality of the physical stresses to which erythrocytes are subjected by the motion of the circulation is familiar to anyone who has observed the flow of blood through small vessels and capillaries under a microscope As with the impact of a glass bead in the in vitro test of mechanical fragility when a red cell is forced by the blood pressure into a capillary of smaller calibre than its own diameter it becomes deformed sometimes to such an extent as to resemble a short sausage If the red cell is nearly spherical the resulting stretching of a normally durable envelope may cause destruction If in addition the membrane of the red cell is physically weak the effect of trauma will be enhanced and it is understandable that the deforming stresses of repeated capillary passages may soon cause its rupture Thus it appears that there are significant resemblances between the immediate mechanisms of red cell destruction in patients with thermal burns and for example those in congenital hemolytic jaundice In both conditions the red cells are primarily defective and exhibit increased spheroidicity osmotic and mechanical fragilities In both also the increased mechanical fragility of the erythrocytes though resulting from different causes is apparently the major determinant of the increased red cell destruction

Conclusions

1 Human blood when heated to from 47 to 65 C showed striking morphologic changes in the erythrocytes progressive division with the development of many new forms of various sizes and shapes especially spheroid cells

2 The appearance of spheroid cells coincided with the development of progressive increase in the osmotic and mechanical fragilities of the erythrocytes without significant increase in red blood cell volume Hemolysis in serum or

plasma was slight unless the osmotic fragility of the red cells increased sufficiently to cause hemolysis in isotonic solution of sodium chloride

3 The effects of heat on the osmotic fragility of the red blood cells were above a critical temperature progressive reproducible and proportional to the temperature and time of exposure

4 The change in the red blood cells produced by heat were irreversible and independent of the nature of the suspension medium whether plasma serum or salt solution

5 In view of these facts it is suggested that geometric considerations involved in the progressive division of initially biconcave discoid cells without significant increase of volume or surface predicate the production of progressively more nearly spherical and consequently more osmotically and mechanically fragile new forms Despite lack of evidence for significant change in the permeability of the cell envelope a direct effect of heat in reducing its ability to resist mechanical trauma cannot be excluded

6 The intravenous injection into dogs of dog red blood cells rendered spheroid and osmotically and mechanically fragile by previous heating resulted in prompt hemoglobinemia and hemoglobinuria with selective removal of the abnormal red cells within a few hours Similar results were observed in a fatal human case of thermal burn

7 The intravenous injection of the centrifuged plasma from heated dog blood did not cause erythrocyte destruction or change in the osmotic or mechanical fragilities of the red blood cells of the recipient dog

8 Red blood cells previously rendered osmotically and mechanically fragile by heating exhibit decreases in volume resembling those of normal red blood cells when placed in hypertonic solutions of sodium chloride Also like normal dog red blood cells heated dog red blood cells increase in volume spheroidicity and osmotic fragility upon sterile incubation at body temperature

9 This potentiality for increase in osmotic fragility may partially explain how human red blood cells though insufficiently increased in osmotic fragility to be hemolyzed by isotonic plasma are nevertheless destroyed after intravenous injection as a consequence of intravascular stagnation in certain tissues However the increased liability of the membrane of such red blood cells to rupture by trauma resulting from the motion of the circulation provides a more likely explanation of the immediate mechanism of the increased red blood cell destruction shortly *after thermal burns*

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THE EVENTS IN THE HEMOLYTIC CRISIS OF HEREDITARY SPHEROCYTOSIS WITH PARTICULAR REFERENCE TO THE RETICULOCYTOPENIA PANCYTOPENIA AND AN ABNORMAL SPLENIC MECHANISM

By William Dameshek M D and Marvin L Bloom M D

HEREDITARY spherocytosis (familial hemolytic jaundice) although a chronic disease of varying severity, at times takes on the aspects of an acute illness The patient previously in fairly good health suddenly experiences malaise vomiting fever and nausea Examination reveals an unusual degree of pallor In severe instances a varying degree of shock is present For these acute episodes the designation of hemolytic crisis has long been used

The cause of the crisis has not been determined If the disease is due simply to the production of abnormal red cells i e spherocytes and their removal by an essentially normal spleen the crisis would have to be explained by a sudden great increase in maldevelopment of the bone marrow red cells This is hardly likely Both Doan[13] and Heilmeyer[20] have postulated that the spleen is largely at fault becoming unusually active during the crisis In a previous report of three cases of crisis occurring successively in members of the same family one of us[6] noted a consistent reduction in leukocytes platelets and reticulocytes It was speculated that this might be due to an unusual degree of splenic activity with inhibitory effects upon blood formation in the bone marrow In a more recent article Owren[38] emphasized the pancytopenia and particularly the reticulocytopenia He suggested that the crisis could not be considered as hemolytic but was actually aplastic and stated that no proof of the hemolytic nature of the crisis was available The possibility that hypersplenic effects might be present was discounted

Our observations in seven cases of hemolytic crisis have led us to conclude that the crisis is due to the combination of (1) a marked exaggeration of the usual hemolytic mechanism with (2) arrested maturation of red cells in the bone marrow induced by a pathologically hyperactive spleen The marrow in crisis shows not aplasia but rather maturation arrest of the nucleated red cells at the most primitive or erythrogone level The dramatic and almost immediate effects of splenectomy with the sudden outpouring of red cells leukocytes and platelets indicate strongly that the marrow is fundamentally normal but that the spleen has exerted marked inhibitory effects

It seems likely that the spleen primarily a passive organ which removes spherocytes selectively at times becomes unusually active thus leading to both excessive

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Aided by grants from the Charlton Fund and the U S Public Health Service

This paper is dedicated to Dr George R Minot whose memorable lectures on the physiopathology of diseases of the blood to our class at Harvard Medical School in 1921 did much to stimulate in me a sustained interest in hematology and in the mechanisms of blood formation and destruction *William Dameshek*

phagocytosis and to hypersplenic inhibitory effects upon the marrow The crisis may become so dangerous to life that emergency splenectomy may be required Following splenectomy no further crises develop

Methods

Hemoglobin For the most part photoelectric colorimeter methods[18] were used utilizing the Cenco and Evelyn instruments Fifteen and six tenths grams per 100 cc of blood were considered as the standard of 100 per cent normality

Serum bilirubin The method of Malloy and Evelyn[27] was used with determination of prompt and indirect types Values of less than 1 0 mg of bilirubin per 100 cc were considered normal

Fecal urobilinogen The average daily urobilinogen output in a four day stool collection was determined by the method of Watson[4]

Reticulocyte and platelet counts These were performed in the same preparations by the method of Dameshek[7]

Free erythrocyte protoporphyrin This was determined by the method of Grinstein and Watson[17] after a period of observation and practice by one of us (M B) in the laboratory of Dr Cecil J Watson University of Minnesota Medical School The number of extractions performed was in direct relation to the amount of fluorescence seen

Plasma or serum iron determinations The method of Kitzes Elvehjem and Schuette[24] was used

Immunohematologic studies Methods for the detection of circulating antibodies including the use of bovine albumin as a diluent were utilized as described by Neber and Dameshek[28] Erythrocyte survival time studies were determined by a modified Ashby technic

Hypotonic fragility The hypotonic fragility was determined in the earlier cases by the method of Daland and Worthley[8] More recently particularly in case B S the photoelectric method of Suess Limentani Dameshek and Dolloff[4] was used

Report of Cases

In 1941 one of us[8] described three cases of hemolytic crisis which occurred within the same family during a ten day period These two brothers and a cousin all of whom lived in the same household had in common a seventy year old grandfather who was known to have congenital hemolytic icterus The latter s son who was the father of the first two patients also had the disease

Case 1 D C M 2nd aged 11 had been known to have chronic jaundice anemia and splenomegaly for several years Mild recurring attacks of fever weakness pallor and increased icterus had occurred about three times annually On April 13 1938 the patient suddenly developed pain and vomiting On the following day jaundice and pallor were noted Physical examination revealed an acutely ill boy evidently in mild shock who showed marked pallor and slight icterus of the sclerae and skin The temperature was 103 5 F the pulse rate 120–140 per minute There was moderate splenomegaly the spleen being felt 3 4 fingerbreadths below the left costal margin

Examination of the blood showed hemoglobin 36 per cent (Sahli) R B C 1 25 M W B C 3800 per cu mm The red cells showed extreme spherocytosis and the average red cell diameter was 5 63 microns On April 16 the red cell count had dropped to 1 01 M and an emergency splenectomy was decided upon The patient was then given large (probably excessive) amounts of intravenous fluids and blood both before and during splenectomy Immediately following operation he developed pulmonary edema and expired

Case 2 R M Jr was a 12 year old boy and the brother of the first patient Like his brother he had been known to have had congenital hemolytic icterus for several years He too had had several episodes (in the spring as a rule) of marked pallor and icterus associated with fever and malaise On April 21 1938 he developed fever malaise headache and left sided abdominal pain On the following day he was dizzy and weak Physical examination revealed a temperature of 103 6 F pulse rate 120/minute the general appearance of acute illness extreme pallor slight jaundice splenomegaly and grave anemia The hemoglobin was 30 per cent (Sahli) and the red cell count 1 22 M The white cell count was 3000 per cu mm Extreme spherocytosis was seen and the hypotonic fragility showed beginning hemolysis at 0 64 per

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Case 4 Mary D S a 37 year old housewife was admitted to the J H Pratt Diagnostic Hospital on April 14 1944 complaining of marked weakness Pallor had been noted since December 1943 and the patient s husband had remarked upon the presence of scleral icterus for about a year She had been asymptomatic however until April 8 1944 At that time she developed malaise generalized arthralgia and a shaking chill

Examination revealed moderate fever jaundice and slight stupor The pallor became increasingly prominent and was associated with increasing weakness On admission to the hospital there was barely perceptible icterus a few petechiae in the region of the uvula and soft palate and slight lymphadenopathy in the axillae The heart showed a forceful apical impulse and appeared somewhat enlarged to left A grade II systolic murmur was heard at the apex and was transmitted to the base of the heart The liver edge was tender and palpable 3 4 fingerbreadths below the right costal margin The splenic edge was felt 3 4 fingerbreadths below the left costal margin The blood showed hemoglobin 5 3 Gm (34 per cent) R B C 1 53 M W B C 6600 reticulocytes 0 3 per cent

TABLE 2 —*Hematologic Data Case 3 (M McN)*

Date	R B C	Hgb	W B C	Platelets	Hypotonic fragility	Serum bilirubin	Reticulocytes	Remarks
	mil / cu mm	%	*thousand / cu mm*	*mil / cu mm*	% NaCl	mg %	%	
4/24/38	1 70	35	5000		0 80–0 56			
4/25/38								
a m	1 98	35	5600					
p m	1 83	31	5000					
4/26/38	1 62	30			0 62–0 50		0 8	300 cc transfusion
4/27/38	2 25	38						Splenectomy + 300 cc transfusion
4/28/38	4 11	65	2100				0 8	
4/29/38	4 04	69	17000				0 3	
5/ 2/38	4 60	78					0 3	
5/ 4/38	4 57	93					0 4	
5 17/38	3 96	71					0 2	
/21/38	4 12	80	13600		0 60–0 16			
/22/38	4 27	78						
9/13/38	4 57	84	9500	1 540			2 3	
2/20/39	4 42	90		1 580	0 68–0 16			
4/17/40	4 46	89	7400	1 471	0 64–0 22		3 7	

Transfusions totaling 900 cc were given without reaction and the fever gradually subsided by the sixth hospital day The reticulocytes rose to 5 4 per cent on the fifth hospital day No abnormal circulating antibodies were found in the serum (salt solution used as the diluent)

Splenectomy was performed on April 21 1944 This was followed by an uneventful recovery The patient was seen last on July 3 1947 at which time she was in excellent health Blood studies were completely normal except for persistent spherocytosis (see table 3) Hemoglobin was 12 9 Gm (83 per cent) R B C 4 22 M hematocrit 38 per cent M C V 90 reticulocytes 0 8 per cent platelets 1 73 M and W B C 12 100 per cu mm The serum bilirubin was 0 3 mg per cent (indirect)

Case 5 H C a 14 year old white boy noted vague malaise about March 10 1946 He complained of severe headache and took a patent medicine containing acetanilid with some relief On March 16 he suddenly developed sharp pain in the right lower abdominal quadrant cramp-like in nature and persisting for several hours On March 1 the patient felt considerably improved but on March 19 an acute episode of vomiting occurred On March 20 his physician noted fever of 104 F a severe nonproductive cough and marked flushing of the skin The patient was admitted to the hospital on that day

cent NaCl hemolysis b ing complet at o 34 p r cent NaCl At th h ight of the crisis it was noted that the reticulocytes numbered only o 4 per cent Three transfusions at four hourly intervals were given on April 23 with a resultant rise in hemoglobin to 70 p r cent and in erythrocyte count to 3 3 M Although clinical improvement was noted on the following morning fifteen hours later the red cell count had dropp d to 2 56 M Splenectomy was then performed The next day sharp increases in both the hemoglobin and erythrocyte levels were noted Upon discharge from the hospital on May 6 the hemoglobin was 98 per cent and the red cell count 4 46 M The subsequent cours has been un ventful (s e table 1)

Case 3 Marjorie McN aged 5 was the cousin of the first two patients and liv d in th sam household Lik the others she had b en known to have congenital hemolytic jaundice and had had several episod s of apparent hemolytic crisis On April 22 1938 she suddenly d v lop d malaise and fever and complained of lower abdominal pain and headache

TABLE 1 —*Hematologic Data Case 2 (R M J)*

Date	R B C	Hgb	W B C	Platelets	Hypot nic f agil ty	Serum bilirubin	Reticulocytes	Remarks
	mil l s/ mm	*%*	*tho nds/ c mm*	*mil lio s/ cu mm*	*% NaCl*	*mg*	*%*	
4/22/38	1 22	30	3000		0 64–0 34	5 1	0 4	
4/23/38								
1 00 a m								500 cc transfusion
4 00 a m								400 cc transfusion
8 00 a m	2 54	47	3600					
10 00 a m								400 cc transfusion
12 00 p m	3 66	69					0 6	
3 00 p m	3 33	70						
4/24/38								
9 00 a m	2 56	68						
11 00 a m								Splenectomy
1 00 p m								500 cc transfusion
4/25/38	5 47	93						
4/26/38	4 67	89						
5 6/38	4 64	98						
5 1 /38	3 72	80	8300	538			1 3	
6/14/38	4 61	89		1 200			1 6	
10/18/38	5 06	102	7300	1 113	0 52–0 26		1 2	
2 20/39	4 83	95	8400	740	0 60–0 04		0 2	
4 17/40	4 78	93	8900	1 061	0 56–0 42	0 9	1 4	

Examination revealed an acutely ill little girl ith marked pallor and slight icterus The temperature was 103 F and the pulse rate 120 p r minute The splenic edge was palpable one fingerbreadth below the left costal margin

The hemoglobin wa 35 p r cent (Sahli) The red cell count vas 1 7 M and th white cell count 5000 per cu mm There was marked spherocytosis and the hypoton c fragility test showed b ginning hemolysis at 0 80 per cent with complete hemoly is at 0 30 per cent On th following morning still at the height of the crisis only 0 7 p r cent reticulocytes were found

T vo transfusions of 300 cc each were given on April 26 and 2 following which spl nect my was performed The spleen veighed 350 grams and show ed extrem congestion of the pulp Convalescence was uneventful On May 4 the red blood cell count was 4 57 M th h moglob n 93 per cent and the white cell count 10 200 per cu mm The subsequent course was characterized by a definite increase in the mean red blood cell diameter and a diminution of sphe ocytosis although an abnormal hyp tonic fragility p rsisted The subs quent cour e has been uneventful (ee table 2)

and a generalized morbilliform eruption had app^ared The spleen was palpabl four to six fingerbreadths below the left costal margin Icterus was not noted The blood examination now showed pancytop nia i e anemia leukop nia thrombocytopenia and reticulocytopenia together with an extreme degree of spherocytosis Actual blood findings were hemoglobin 6 0 Gm R B C 2 40 M W B C 3 850 reticu locytes 0 1 per cent

On the basis of the above findings the diagnosis of congenital hemolytic anemia in crisis was made Splenectomy was p rform d after four transfusions had b en given (two on the day of admission one on

TABLE 5 —*Hematologic Data Case 6 (P O N)*

Date	R B C	Hgb	W B C	Pl t lets	Hypot c f g l ty	S rum b l b	Fec l u bil og	Ret c l cytes	Remark
	mil / mm	*Gm*	*th s nd / c mm*	*mil lio s/ c mm*	*% NaCl*	*mg %*	*mg / d y*	*%*	
10/30/45	2 61	9 1	9000	0 213	0 52–0 34	1 8			
11/10/45	2 72	9 1	8250		0 56–0 30	2 0			
11/23/45					0 56–0 40			6 4	
11/28/45	2 88	10 4		493	0 52–0 38			10 2	
12/ 5/45								9 3	
12/21/45	2 82	11 0	9450		0 60–0 24			9 2	
1/ 9/46	2 91	11 0						9 1	
4/ 8/46	3 20	10 3	9000	810	0 56–0 36	1 4		7 7	
5/ 4/46						1 1			
5/20/46	3 45	12 0	4550		0 60–0 36	1 4			
5/27/46	3 89	11 0	6900				120		
6/ 3/46	3 76	12 3	5100	330	0 60–0 34	2 0		6 9	Beginning of minor crisis
6/ 5/46	3 24	12 3	3300			1 7		7 6	
6/ 7/46	3 59	12 7	5350		0 46–0 36	3 3		7 6	
6/10/46	3 21	10 7	6200		0 68–0 36	1 4		8 4	
6/21/46	3 48	11 7	5350			2 0	1050	7 8	
6/27/46	3 33	11 0	4650		0 46 0 34	1 5		8 1	
7/ 3/46	3 6	12 5	5000					7 8	
7/10/46									Splenectomy + 1000 cc blood
7/11/46	5 45	15 1	16900						
7/12/46	4 9	15 1	14000		0 64–0 36	1 2			
7/16/46	4 4	14 1	8200		0 68–0 36				
8/ 5/46	4 1	12 5	7200	500				0 8	
10/21/46	4 3	12 5	6750	443				0 6	
6/ 4/47	4 5	13 3	6550	907		1 0		1 2	

the thi d day and another just prior to operation) The spleen was greatly enlarged and at least six accessory spl ens were present The course after splenectomy was uneventful this was particularly striking in vie v of the very critical condition of the patient before operation

Case 6 P O N Thi 26 ye r old woman v as first seen in O tober 1945 The patient came from a known family of f milial hemolytic jaundice and represented the fourth generation in vhich the disease had been known [45]

Intermittent ja ndice h d been noted first when she vas 15 Ho vever she seemed to be in good health until 1943 a few months fter the de th of her father of severe hemolytic crisis at the age of 55 At that time she was two month pregnant She developed chills fever and became ve y pale The quest on of mala ia was considered but mal ial parasites were not found The pregnancy proceeded uneventfully

On admission he was acutely ill and appeared weak flushed and slightly cyanotic He was apathetic and moderately pale The oral mucous membranes appeared to be edematous and spongy Scattered rales were heard at the base of the left lung The heart rate was rapid but there was no evidence of enlargement and no bruits were heard Marked tenderness was elicited in the lower quadrant of the abdomen particularly on the right The spleen seemed soft and was palpable four fingerbreadths below the left costal

TABLE 3 —*Hematologic Data Case 4 (M D S)*

Date	R B C	Hgb	W B C	Platelets	Serum bilirubin	Reticulocytes	Remarks
	millions/c mm	*Gm*	*thousands/cu mm*	*millions/c mm*	*mg %*	*%*	
4/14/44	1 53	5 3	6600	084	2 3	0 3	
4/15/44	1 90					0 2	
4/17/44	2 06	7 6	6600	188		1 9	
4/18/44	2 31	8 2	8400	268		5 4	
4/20/44	2 95	8 3	4200				
4/21/44							Splenectomy
4/22/44	3 75	10 5	23300				
4/25/44	3 35	10 4	15000				
4/27/44	3 36	10 6	13200				
5/18/44	4 32		16400				
4/13/45	5 03	14 4	14500				
6/20/46	4 35	14 6	16,00	705	0 8	0 5	
7/ 3/47	4 22	12 9	12100	1 732	0 3	0 8	

TABLE 4 —*Hematologic Data Case 5 (H C)*

Date	R B C	Hgb	W B C	Icterus index	Reticulocytes	Remarks
	mill /c mm	*Gm*	*thous nd /c mm*		*%*	
3/20/46	3 10	8 9	9250	30		
3/21/46	2 40	6 0	3850	20	0 1	
3/22/46	2 8	7 7	5000			
3/23/46						Splenectomy
3/25/46	3 8	10 6	22000			
3/26/46	3 9	9 7	30000			
3/27/46	4 7	10 7	40000			
3/28/46	4 2	10 9	35500			
3/29/46	4 0	11 6	20950			
3/30/46	4 3	10 9	24000			
4/ 1/46	4 4	11 6	17800			
4/ 3/46	4 4	11 9	17300			
4/ 5/46	4 3	11 8	13800			

margin It was extremely tender and there was some spasm of the overlying abdominal muscles A questionable bilateral Kernig reflex was elicited there was a suggestion of a bilateral Babinski reflex and meningismus was present Blood studies showed hemoglobin 8 9 Gm R B C 3 10 M W B C 9 250

The question of meningitis was considered seriously on the first day of admission It was found that the patient had a rapidly progressive anemia Penicillin therapy was not attended by improvement On March 21 generalized lymphadenopathy was found The nodes were bean sized and nontender A few bleeding areas were noted in the oral mucous membranes petechiae were present over the anterior chest

Jaundice was not a prominent feature during the hospital course Very slight scleral icterus was noted during the first two hospital days after which it disappeared Transfusions of 500 cc 400 cc and 500 cc of freshly drawn and citrated blood were administered on the first second and third hospital days On the second hospital day there was slight improvement and following the third transfusion the patient felt considerably better At the outset microspherocytosis dominated the blood smear The reticulocytes were conspicuous by their complete absence during the first four hospital days despite the severity of the anemia and the hemolytic process On the fifth hospital day 0 1 per cent reticulocytes were found and then a gradual rise took place to a peak value of 12 6 per cent on the twelfth hospital day At no time during the fourteen days of hospitalization did the red blood cell count or hemoglobin concentration approach normal values Nevertheless when she was discharged on March 7 the patient stated that she felt as well as she ever had At that point the following blood counts were present R B C 2 76 M hemoglobin 8 3 Gm (53 per cent) reticulocytes 10 4 per cent

The patient was followed at frequent intervals and was then readmitted for splenectomy which was performed on April 16 1947 by Dr C Stuart Welch Just before the operation the blood counts were as follows R B C 2 61 M hemoglobin 8 7 Gm (56 per cent) reticulocytes 10 4 per cent The postoperative course was uneventful

On June 23 1947 the patient was asymptomatic and appeared to be in excellent general health At this time the blood counts were normal (R B C 4 66 M hemoglobin 14 6 Gm) When seen on September 5 194 the patient was in excellent health and had gained considerable weight Her mother stated that she had never been so well (see tables 6–10 figures 1 8)

Analysis of Data

The Blood Picture

In the report[8] from this laboratory in 1941 the following comment was made The reduction in leukocytes thrombocytes and reticulocytes in the cases reported here appears at first glance somewhat unusual since in the presence of an acute hemolytic process one would expect to find the evidences of increased regenerative activity on the part of the bone marrow that is leukocytosis thrombocytosis and reticulocytosis As further observations augment experience with the hemolytic crisis in our own cases as well as in those reported in the literature it becomes apparent that pancytopenia and reticulocytopenia are usually present

In case 7 no reticulocytes (figure 5*a* table 6) whatever were found at the height of the crisis In fact it was not until the fifth hospital day that the finding of 0 1 per cent reticulocytes was recorded A gradual reticulocytosis then occurred reaching an initial peak of 12 6 per cent on the thirteenth day of observation Thereafter reticulocytosis was sustained until splenectomy was performed on the fifty fifth day of observation At the height of the crisis there was also a moderate thrombocytopenia and granulocytopenia (23 per cent segmented and 7 per cent band forms) although the total white cell count was 15 500 per cu mm By the fifth hospital day when reticulocytes were finally seen the thrombocytopenia and granulocytopenia had disappeared Thereafter no significant changes in the white cells or platelets were noted until the postsplenectomy period when the expected thrombocytosis and granulocytosis occurred The platelets have continued at unusually high levels

The opportunity for such detailed studies was not afforded in the other cases most of whom were seen under less ideal conditions for investigation In general however these patients pursued a course quite similar to that of the seventh case Thus in case 1 anemia and leukopenia were present at the height of the crisis

although a p rsistent yellowish tint of the sclerae continued She complain d of fatigue recurring dizziness and som dyspnea on exertion

Examination at this time during the sixth month of her second pregnancy elicited slight scleral icterus moderate splenomegaly a systolic murmur over the precordium minimal pitting edema of the ankles and pretibial regions and som superficial varicosities of the lower extremities Examination of the blood showed R B C 3 10 M hemoglobin 65 per cent W B C 12 500 and marked spherocytosis with increas d polychromatophilia and reticulocytes The pregnancy was uncomplicated by any untoward incidents and a normal child was delivered on Januray 23 1946 There was however continued fatigue and int rmittent exacerbations in the chronic icterus Slight pallor icterus and splenomegaly continued to be the outstanding physical findings Representative serial laboratory findings are recorded in table 5

Case , The present study was initiated as the result of observations in this case and most of the illustrative figures are based on the data obtained

Beverly S a 13 year old white female of Portuguese origin one of nonidentical twins entered the J H Pratt Diagnostic Hospital on February 21 1947 She complained of fainting spells weakness chills and fever of three days duration About a week prior to admission she was stated to have contracted a mild upp r respiratory infection Four days before admission her mother noted pallor On the following day the patient complained of dizziness marked weakness and severe headache The temp rature rose to 104 F that evening She vomited greenish bitter material and had severe vertigo Nausea and vomiting persisted and on the day of admission she was dizzy and very weak

About one month before this admission there had been an episode of right lower quadrant pain un associated with nausea or vomiting Although appendicitis was suspected surgery was not performed A week b fore admission another similar episode of abdominal pain occurred shortly after the onset of the last m nstrual period This subsid d quickly Several days before admission the local physician remarked upon th fact that the patient was jaundiced Neither dark urine nor light stools had been seen

It was not possible to elicit any history of familial jaundice or anemia either in the parents grand *parents close relatives or in the five siblings* *

The patient had always been noted to b considerably paler than her tw in brother She was a high school student and gave no history of exposure to chemicals or drug ingestion Fava beans had been eaten occasionally by the family but not for about six months The past history was irrelevant

Examination on admission revealed an exceedingly well developed and well nourished girl who appeared very apathetic and critically ill bordering upon shock She was drowsy and hardly able to respond to simple questions There was marked pallor and slight scleral icterus The temperature was 100 F puls 120/minute and blood pressure 105/50 The examination of the chest was negative *The* spleen was readily palpable two to three fingerbreadths below the costal margin The liver was not en larged and there was no tenderness in the right upper abdominal quadrant Representative blood finding are ho vn in table 6 In brief at the time of admission they showed hemoglobin 4 0 Gm (<25 per cent) R B C 1 3 M/cu mm reticulocytes 0 0 per cent ( ) W B C 15 500 per cu mm

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* Inasmuch as no evidenc could be adduced in this cas for the diagnosis of familial or heredi ary spherocytosis the reasons prompting our diagnosis of congenital spherocytosis deserve mention

Detailed study of the parents siblings and relatives was difficult becaus the patient came from a small town (Provincetown) situated more than 100 miles from Boston

Through the cooperation of the patient s family physician Dr Thomas Perry of Provinceto vn it was found that her father twin brother and the other two siblings showed no abnormal physical findings Blood smears which were for arded to us for examination were within normal limits The mother was examined carefully but no significant physical or hematologic findings were elicited

That the disease may be congenital and not familial has been noted among others by Wolman[54] and Race [40] The latter observer found one affected child in each of three families although neither the parents nor the siblings nor the close relati es exhibited any evidence of the disea e

Lack of immune iso-antibodies in the blood serum excellent clinical response to splenectomy the persistence of spherocytosis the normal s irvival time of transfused red cells before splenectomy and the complete lack of any e idence indicating an acquired hemolytic process of well defined etiology tended to support the initial clinical impression of an hemolyt c crisis occurring in the course of congenital hemolytic jaundice even though evidence of familial disease was entirely lacking

Jaundice was not a prominent feature during the hospital course Very slight scl ral icterus was noted during the first two hospital days after which it disappeared Tran fusions of 500 cc 400 cc and 500 cc of freshly drawn and citrated blood were administered on the first second and third hospital days On the second hospital day there was slight improv ment and following the third transfusi n the patient f lt considerably better At the outs t micr spherocyto is dominated the blood smear The reticulocytes were conspicuous by their complete absence during the first four ho pital days d spite the s verity of the anemia and the hemolytic process On the fifth hospital day o 1 per cent reticulocytes w re found and then a gradual rise took place to a p ak value of 12 6 p r cent on the twelfth ho pital day At no tim during the fourteen days of hospitalization did th red blood c ll count or hemoglobin concentration approach normal values Nevertheless when she was discharged on March — the patient stated that she f lt as well as she ever had At that point the following blood counts were present R B C 2 6 M hemo globin 8 3 Gm (53 per cent) reticulocytes 10 4 per cent

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The opportunity for such detailed studies was not afforded in the other cases most of whom were seen under less ideal conditions for investigation In general however these patients pursued a course quite similar to that of the seventh case Thus in case 1 anemia and leukopenia were present at the height of the crisis

TABLE 6 — *Representative Blood Counts Case 7 (B S)**

Day of observation	Date	R.B C	Hgb	W B C	Granulocytes (segmented forms)	Granulocytes (band forms)	Platelets	Reticulocytes	Remarks
		millions/cu mm	*Gm*	*thousands/c mm*	%	%	*millions/c mm*	%	
1	2/21/47	1 3	4 0	15500	23	7	0 35	0 0	Transfusion
2	2/22/47	1 8	4 6	8400	36	9	0 34	0 0	Transfusion
3	2/23/47	2 1	4 9	7600	57	11		0 0	Transfusion
4	2/24/47	2 3	6 6	11100	55	16	0 45	0 0	
5	2/25/47	2 5	7 4	9900	57	10	0 50	0 1	
6	2/26/47	2 6	6 6	10800	47	7	0 52	3 6	
7	2/27/47	2 3	6 2	9800	52	6		4 4	
8	2/28/47	2 5	7 4	8400	35	7	0 50	4 2	
11	3/ 3/47	2 7	7 8	9600	52	4	0 50	7 9	
12	3/ 4/47	2 5	8 5	6800	59	3		12 1	
13	3/ 5/47	2 7	8 5	11300	57	12	0 53	12 6	
14	3/ 6/47	2 6	7 5	11300	70	1		11 2	
15	3/ 7/47	2 8	8 3	14100	68			10 4	
21	3/13/47	2 8	8 7	12100	62	3	0 48	16 3	
32	3/24/47	2 6	8 7	8700					
49	4/10/47	3 3	8 8	12100	57	8	0 48	14 6	
51	4/12/47	2 9	9 3	10200					
53	4/14/47	3 1	8 8	10600	68		0 65	17 2	
54	4/15/47	3 0	9 3	8100	64	5		22 6	
55	4/16/47	2 6	8 7	8100	65	4	0 76	22 1	
55	4/16/47	3 1	9 3	29100					Splenectomy
56	4/17/47	3 6	8 7	18800	85	4	2 1	27 6	
57	4/18/47	3 4	8 3	19400	75	8			
58	4/19/47	3 5		17200	75	7	1 8	25 0	
59	4/20/47	3 4		14100	54	8	1 8	25 0	
60	4/21/47	3 5	8 7	9100	67	4	2 3	29 3	
61	4/22/47	3 4	9 3	8100	55	4	1 8	23 8	
62	4/23/47	3 3	9 5	8500	55	6	- 3	24 2	
63	4/24/47	3 3	9 5	8100	71	2			
64	4/25/47	3 7	10 4	6200	41	3	2 3	11 2	
65	4/26/47	3 8	10 7	12100	59	4	2 6	2 9	
67	4/28/47	3 7	11 0	10100	63	2	2 7	3 8	
68	4/29/47	3 8	11 0	9100	62	1	2 7	3 9	
69	4/30/47	3 6	10 7	7700	48		2 5	4 6	
4	5/ 5/47	3 6	11 0	6800	45	3	1 9	3 0	
76	5/ / 4	3 7	11 0	9600	59	2	1 8	3 1	
78	5/ 9/47	3 7	10 7	7900	52		1 9	2 8	
100	5/31/47	4 4	12 1	11700	56	5	1 5	0 5	
123	6/23/47	4 7	14 6	8100	57		2 3	2 1	
197	9/ 5/47	4 4	13 3	8200	51	6	1 1	1 2	

The first day of observation coincided with the height of the hemolytic crisis Transfusions of 500 cc 400 cc and 500 cc of freshly drawn and citrated blood were administered on the first second and third hospital days Splenectomy was performed on the fifty fifth day of observation

In case 2 anemia leukopenia and reticulocytopenia (0 4 per cent) were present The postoperative period was characterized by a great outpouring of red blood

cells and platelets unfortunately reticulocyte and other blood cell counts were not available for adequate comparison In case 3, reticulocytopenia (0 7 per cent) anemia and leukopenia were present during the crisis Following splenectomy the red blood cell count rose precipitously and leukocytosis occurred but no parallel reticulocytosis was seen In case 4 reticulocytopenia (0 3 per cent) was present during the crisis in addition there was marked anemia thrombocytopenia and a

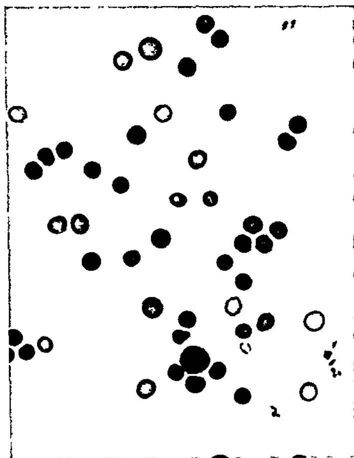


Fig 1 Case 7 (B S) Peripheral blood at the height of crisis showing the predominance of mircospherocytes

normal or somewhat leukopenic white cell count of 6 600 By the fourth day a slight reticulocytosis of 5 4 per cent was present and thrombocytosis was developing

Splenectomy was followed by a sharp rise in erythrocyte and leukocyte counts but the data regarding reticulocytes and platelets are inadequate In case 5 anemia leukopenia and reticulocytopenia (0 1 per cent) were present during crisis and the blood platelets were definitely reduced on smears In case 6 during a minor hemolytic crisis the reticulocytes were increased to about 7 per cent but there was a definite leukopenia of between 3300 to 5300

Extreme spherocytosis was an outstanding feature of the crisis in all our cases There was very little evidence of the biphasic type of blood cell picture seen when large polychromatophilic reticulocytes are also present The picture is strikingly similar to that seen in the acute hemolytic anemia produced experimentally by large doses of hemolytic antiserum [8]

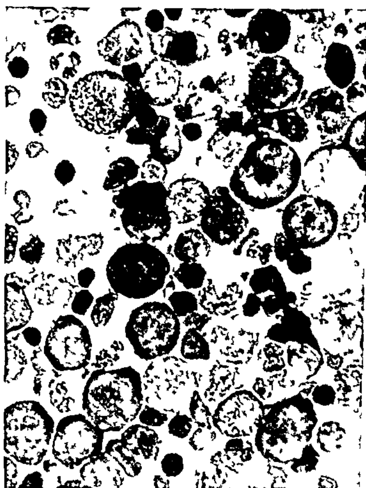


Fig 2 Case 7 (B S) Bone marrow at the height of crisis showing the accumulation of primitive erythroblasts (pronormoblasts erythrogones) and lack of maturation The small mononuclear cells are lymphocytes

In case 7 the Price Jones curves (figure 6 table 8) of the red cells at the height of the crisis (and before transfusion) demonstrated that about 50 per cent of the red blood cells had a diameter of 4 8 micra or less almost all the red cells had a diameter of less than 7 0 micra Seven weeks later and just prior to splenectomy 37 per cent of the red cells were 6 4 micra in diameter and 25 per cent of the cells had a diameter of 7 2 to 8 8 micra Five days after splenectomy and probably due to an additional release of reticulocytes into the peripheral blood there was a definite secondary peak at 8 0 micra This latter figure represented the diameter of 22 per cent of the cells although over 20 per cent still had a diameter of 5 6 micra

TABLE – —Bere ly S Spees l St dies

D te	D y of Ise t n	Fec l u b l nog	Hypt f g l ty	Serum b l rub n
		mg/d		mg %
2/22/4	2		c 0 40	
2/24/47	4			1 1
2/26/4	6			1 –
3/ 5 4	13	255		
3/ –/4	15			1 0
3/13/4	21		0 66 0 39	
3/24/4	3		0 69 0 42	
3/26/4	34		0 64–0 36	
4/10/4	49			
4/15/4	54			3 4
4/21/4	60			1 3
4/22/4	61		0 2–0 39	
4/25/47	64		0 2–0 40	
4/28/47	6			1 –
4/30/47	69	35		
5/ 5/47	74			0 8
5/ 9/47	–8			0 9
5/31/47	100	41		0 9
6/23/47	123			



FIG 3 CAS 7 (B S) Peripheral blood five days follo ving the h ight of the crisis Demonstrating the appearance of increased number of larger red blood cells and the relative or absol te decrease in microsphe ocytes

or less Five months after splenectomy the Price Jones curve showed a main peak (39 per cent of cells) at 6 4 micra with a wide base due to the persistence of sphero cytes

That the small red cells are truly spherocytes is borne out not only by their microcytic character and their abnormally dense appearance in the stained blood smear but by their greatly decreased resistance to hypotonic solutions of sodium



FIG 4 CASE 7 (B S) Bone marrow fiv days following the h ight of the crisis Active progressive maturation of the normoblastic series is demonstrated

chloride All our cases of hemolytic crisis showed a greatly increased fragility Occasionally slight hemolysis has even been noted in concentrations of sodium chloride solution approaching that of normal saline Extensive studies of hypotonic fragility were made only in case 7 (tables 1-4 7 figure 7) in which the determina tions were carried out by means of our newly devised photoelectric method [41] By this method curves of hemolytic increments somewhat similar to Price Jones curves are obtained These indicate graphically the different types of red cell population according to their thickness variation

The blood picture immediately following splenectomy showed a dramatic increase in all the cellular elements In fact the rapidity of increase of the red cells white cells and platelets suggested a sudden outpouring of these cells from the marrow to the blood (figure 9)

Bone Marrow Picture

Studies of the marrow by aspirations were performed in cases 2 3 4 6 and 7 The relative time of marrow aspiration differed in these cases As already mentioned the conditions for study were ideal only in case 7 However in case 2 marrow was obtained at the height of the crisis and showed a maturation arrest type of erythropoiesis closely similar to that seen in case 7

Frequent aspirations of the sternal marrow both during and after crisis were carried out in case 7 At the height of the crisis when no reticulocytes were found

TABLE 8 —*Representative Erythrocyte Diameters in Case 7 (B S)*

Diameters in microns	2/21/47	4/14/47	4/21/47	9 /47
	F t m at h ght f cr b f e t a l	Two days prior to splenectomy	Five days following splenectomy	Five months following splenectomy
	%	%	%	
3 2	0 8	0	0	0
4 0	9 6	0	0	0 4
4 8	39 2	18 0	8 4	9 2
5 6	31 6	20 0	13 6	18 0
6 4	15 2	36 8	34 0	38 8
7 2	2 0	12 0	20 0	18 4
8 0	1 6	12 4	22 4	14 8
8 8		0 8	1 6	0 4

Marked microcytosis during crisis indicating extreme degree of spherocytosis and increase in size of red cells prior to splenectomy after termination of crisis

in the peripheral blood the marrow preparations showed increased cellularity (figure 2) Granulocytopoiesis was active and the megakaryocytes were normal both in number and in platelet production However there was a striking abnormality of erythropoiesis indicated by a complete lack of mature orthochromatic normoblasts (Normoblasts C) (figure 2) The great majority of the nucleated red blood cells were of the primitive variety i e erythrogones or pronormoblasts There were only small numbers of basophilic and polychromatophilic normoblasts (types A and B) (see table 9 figures 1 and 2)

From these observations it was apparent in this case that there was a distinct maturation arrest of the erythropoietic tissue at or just beyond the erythrogone (pronormoblast) level On the fifth day coincidentally with the appearance of a few reticulocytes another sternal puncture was performed This showed a normoblastic erythropoiesis with adequate numbers of polychromatophilic and orthochromatic normoblasts indicating that the arrested maturation had run its course (table 9 figures 3 and 4) In four subsequent marrow aspirations erythropoiesis continued to be hyperactive and no further evidence of maturation arrest was seen

Immunohematologic Findings and Erythrocyte Survival Time

Studies of the serum for immune bodies were performed in cases 1 to 4 and in case 7 In the first four cases, immune bodies were searched for using normal salt solution as a diluent but in none of these cases were abnormal agglutinins or hemolysins discovered in the serum In cases 6 (P O N) and 7 (B S) iso anti bodies were discovered with the use of bovine albumin solution as a diluent, whereas negative results had been obtained using salt solutions In case 6 (P O N) an abnormal autohemolysin and isohemolysin reacting best at 37 C was discovered with the use of bovine albumin as a diluent disappearing following the termina tion of the crisis The survival time of transfused red cells was studied before and after splenectomy (Figure 8*a*) The curve of red cell disappearance before splenec tomy was definitely abnormal and exponential in type indicating that the abnor mal antibody present was capable of destroying all types of red cells indiscrimi nately including the patient s Following splenectomy the survival time became normal again coincidentally with the disappearance of abnormal antibody

TABLE 9—*Bone Marrow Studies Differential Cot nt of Nu leated Red Blood Cel's in Case 7 (B S)*

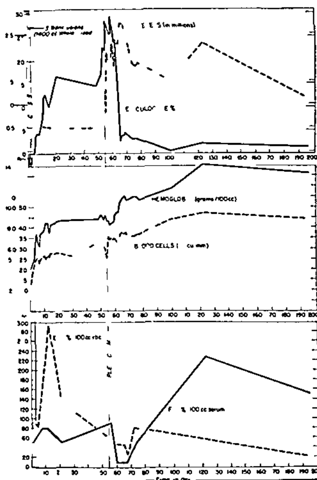
Date	Cra locyte eryth obl t (G E rat)	Erythrogones pronorm bla t	Basoph l c ormobl t (A)	Poly h omato- ph l rmoblast (B)	O thochrom t rmobla t (C)
		%	%	%	%
2/22/47	3 1	79	20	1	0
2/27/47	1 2	3	8	48	41
3/ 5/47	1 1	7	11	55	27
4/17/47	1 5 1	7	19	62	12
4/25/47	1 5 1	4	8	40	48
9/ 5/47	1 5 1	4	26	59	11

The blood group of case 7 was B Rh positive Almost immediately following admission she was given a transfusion of 500 cc of fresh group O Rh positive blood with added A and B group specific substances (Witebsky) On the second hospital day a transfusion of 400 cc of fresh group O, Rh positive blood was given with added A and B (Witebsky) group specific substances During the third hospital day the last transfusion i e of 500 cc of fresh type B Rh positive blood was given No reactions occurred with any of the transfusions The survival time of the trans fused Group O red blood cells was studied by the Ashby technic[36] and found to be normal i e 120 days (figure 8*b*) No transfusions were given in connection with the splenectomy and the disappearance of the transfused cells did not appear to be affected by the splenectomy (see figure 8*b*) Thus the two cases of hemolytic crisis differed radically with respect to red cell survival time perhaps indicating some what different mechanisms

Bilirubin and Urobilinogen

Jaundice was not an outstanding feature of these cases in crisis The available data concerning serum bilirubin have been tabulated with the other blood studies in each case (see tables 1 and 7)

Determinations of the fecal urobilinogen were performed only in case 7 Due to various technical difficulties this patient s stools were unfortunately discarded during the first eight days of her hospital stay A four day stool collection was obtained during the succeeding period when the hemoglobin varied between 7 8 and 8 5 grams per 100 cc At this time the fecal urobilinogen excretion was 255



F G 54 CASE 7 (B S) Graphic representation of t vo hundred day period of observation shov ing the variations in the significant serial studies The lower graph describes the fluctuations in erythrocyte protoporphyrin and serum iron values

milligrams per day This is a definitely high value when related to the hemoglobin concentration corresponding roughly with the excretion in a normal girl of about 500 mgs per day (normal 100–150 mgs per day) This indicated an approximately threefold increase in blood destruction at that time Since the patient was improving at that time it seems reasonable to assume that the fecal urobilinogen output during the first eight days may have been even greater The two determinations of fecal urobilinogen obtained during the postsplenectomy period were within normal limits

Erythrocyte Protoporphyrin

The normal range of concentration of free protoporphyrin in the intact circulating erythrocyte has been determined by Grinstein and Watson[17] to be between 15 and 40 gammas per 100 cc of red blood cells the usual normal value is in the vicinity of 30 gammas per cent or lower

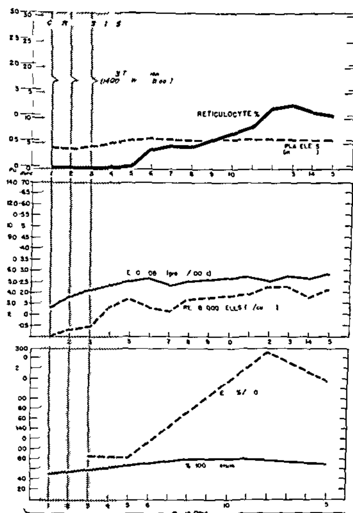


FIG 5*b* CASE 7 (B S) Close up of certain values in the first fifteen days of the clinical course

Serial determinations of free erythrocyte protoporphyrin (EP) were obtained only in case 7 (table 10 figure 5) At the height of crisis at a time when reticulocytes were completely lacking the EP was 87 8 gammas per cent well above normal limits This was perhaps due to an increased erythropoietic activity just prior to the development of the crisis As recovery from the crisis progressed and reticulocytes appeared in the blood (coincident with marked marrow erythropoiesis) the EP concentration rose rapidly reaching peak levels of 292 gammas per 100 cc On the twelfth day of observation the EP gradually decreased until on the sixty

eighth day it reached a normal value of 25 gammas per cent Following this it was found that a state of hypoferremia had developed This was mirrored soon later by a definite increase in the EP Following the administration of ferrous sulfate the EP fell again to within normal limits

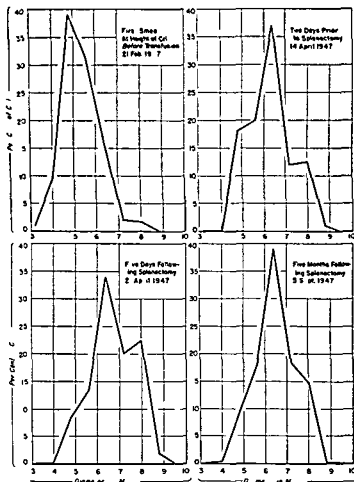


FIG 6 CASE 7 (B S) Price Jones curves of representative blood smears during the clinical cours

Serum Iron

The concentration of serum or plasma iron and its normal range has been defined by several investigators[31 3 39 47 48 5] as ranging between approximately 50–180 gammas per 100 cc serum or plasma

In case 7 B S a serum iron determination at the height of the crisis was 50 gammas per 100 cc concentration i e at the lowest range of normality (see table 10) The patient was not given iron therapy at this time her only sources of iron being through an unrestricted diet and three transfusions After the transfusions had been given a value of 80 gammas per cent was obtained During the postcrisis

(presplenectomy) period this comparative plateau of serum iron values seemed to have no correlation with the great fluctuations in free erythrocyte protoporphyrin

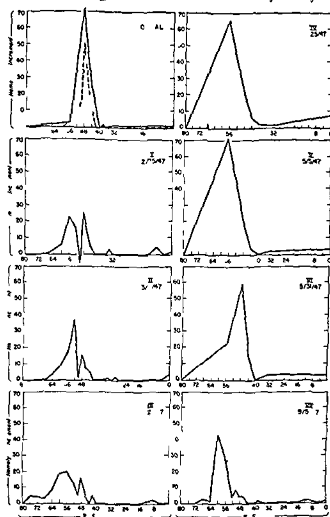


Fig 7 Case 7 (B S) Curves of Hypotonic Fragility

Two methods were used to determine the curves illustrated above These differed only in detail One involves the use of 29 tubes containing 29 serial saline dilutions from 0 80 to 0 0 per cent NaCl In each of these the per cent hemolysis was determined using the Evelyn photoelectric colorimeter The other uses only 7 tubes containing the dilutions which have been found to be most critical 0 80 0 56 0 48 0 44 0 40 0 32 and 0 0 per cent The graph of the normal fragility shows curves from both of these methods the solid line indicating 29 dilution and the dotted line 7 dilutions Graphs I II III and VII depict 29 tube studies whereas IV V and VI show curves from the 7 tube test

The hemolytic increment means simply the change in degree of hemolysis from tube to tube as hypotonicity increases (from above downwards) Therefore at each point of changing saline concentration the per cent hemolysis is obtained by the Evelyn photometer this is compared with the per cent hemolysis in the previous tube and the per cent difference or the hemolytic increment is obtained

However two days following splenectomy a value of 90 gammas per cent was obtained that this was followed by a sharp drop of the serum iron value to 10 gammas per cent (marked hypoferremia) At this point the EP values had reached

normal limits and it was predicted that on the basis of the hypoferremia the EP concentration would rise This actually occurred (table 10 figure 5a)

Ferrous sulfate was then given and a significant rise in serum iron occurred It is possible that the drop of the serum iron value was due to the continued deple tion of the depot iron stores by the persistent demands of hyperactive erythropoiesis coupled with menstrual iron loss

Discussion

The Hemolytic Crisis of Hereditary Spherocytosis

Familial spherocytosis is characterized by a chronic hemolytic anemia of variable severity in which spherocytosis and increased hypotonic fragility are prominent features

The chronic course of the disease is often punctuated by minor and major ex acerbations in the intensity of the hemolytic process Minor episodes are charac terized by malaise low grade fever increased pallor and icterus These episodes last a few days or a week subside spontaneously and quickly and in fact often go unrecognized

The major exacerbations may be so severe as to endanger life Beginning like an acute febrile illness they progress rapidly with the development of abdominal discomfort marked pallor dizziness nausea and vomiting chills and fever Diarrhea may occur and discharges of bile and grossly bile stained stools may be present During the height of the crisis the patient may go into shock or a shock like state stupor and syncope are common Examination reveals an extremely sick looking person who is markedly pale but only slightly jaundiced The pulse is rapid and feeble and the blood pressure is often low with a low pulse pressure The spleen often becomes considerably enlarged as compared with its previous size and is frequently tender

The management of the crisis has been described in a previous paper [6] The use of *fresh* whole blood is of paramount importance Transfusions not only add red cells but have a well defined effect on the blood (and plasma) volume The sudden lowering of the red cell count to levels of 3 5 M to 1 5 M may result in the symptoms of shock Therefore appropriately spaced transfusions may be life saving It should be noted however that transfusions probably have little if any effect on the mechanism of the crisis itself and may well be overdone (Overtransfusion was probably responsible in large part for the fatal determination in case 1) By the judicious use of transfusions and intravenous fluids an adequate prepara tion of the patient for splenectomy is possible This type of management results in a quieter and less toxic patient and thus in a far better surgical risk and a smoother convalescence

Another remarkable feature of the hemolytic crisis is its occurrence in rapid suc cession in several members of the same family Scott[1] reported in 1935 the serial onset of acute blood crises in an entire family In Dedichen s series [12] reported in 1937 13 members of 2 neighboring families living in a small town in Norway developed crises within a few days of each other One of us[6] in 1941 reported three cases of familial crisis occurring within ten days In 1945 Horne Lederer

Kirkpatrick and Leys [1] reported hemolytic crisis in the mother and four children of a family of eight and also in the mother s cousin who lived nearby In these cases too, the illness developed in rapid succession in one individual after the other *A few days as a rule elapsed between the onset of the crisis in the various cases*

The occurrence of the crisis in several members of one family and in two instances in neighboring families, has naturally led to investigation for an extrinsic cause for the crisis Thus in Dedichen s large series,[1] it was suspected that some sort of highly contagious respiratory infection was responsible at least for initiating the crisis This appeared to be substantiated by the development of an acute febrile illness in one of the siblings who did not have the congenital hemolytic disease and during the period when the others were having hemolytic crises Horne *et al* [1] placed a ferret in the home of their first family to develop crises in the belief that a virus infection such as influenza might be the responsible agent The ferret was returned to the laboratory and died shortly thereafter with symptoms of a nasal discharge and conjunctivitis

The occurrence of the crisis in families with incubation periods of a few days between cases is strong presumptive evidence that an infection is responsible for at least initating or precipitating the crisis However since no real proof of an infectious etiology has been adduced it is also possible that no infection is present but that fever rapid pulse headache malaise nausea and vomiting are the results of rapid blood destruction alone

A final feature of the hemolytic crisis is that it occurs only in the presence of a spleen i e following splenectomy crises either do not occur or are extremely unusual This is another indication of the importance of the spleen in initiating crisis (cf below)

Lowe [6] who studied a case during crisis in which the red cells hemoglobin and reticulocytes continued to diminish despite therapy concluded that suppression of marrow function might be responsible Unfortunately studies of the marrow were not performed In the cases of Horne *et al* [1] mentioned above in which complete lack of reticulocytes was reported at the height of the crisis a marrow examination was performed in only one case and then on the eleventh day of illness when the patient was improving These authors referred however to a statement by Josephs [3] who believed on theoretical grounds that a depression of marrow activity might be vital to the pathogenesis of the crisis

Owren[38] in a recent comprehensive article reported crises in six cases of congenital hemolytic jaundice and also stressed the occurrence at the height of the crisis of leukopenia thrombocytopenia and reticulocytopenia He was able to study one case before during and after crisis and obtained serial examinations of blood and marrow Owren concluded that there was an erythropoietic aplasia during crisis He believed that the sudden drop in red cell count occurring in crisis could be adequately explained on the basis of an aplastic reaction on the part of the erythropoietic tissue and went so far as to deny that the factor of hemolysis played any role whatever in the crisis He suggested that the term hemolytic for the crisis be dropped

Serial punctures of the marrow in case 7 indicated that the marrow reaction passes through several distinct stages which could be correlated with the reticulocyte picture During the height of the crisis when reticulocytes were lacking the marrow (figure 2 table 9) showed a nucleated red cell population consisting almost entirely of the most primitive cells i e erythrogones (pronormoblasts) and some basophilic normoblasts Several days later and simultaneously with a slight increase of the reticulocytes in the blood there was a complete reversal (figure 4 table 9) in the marrow picture Most of the nucleated red cells were of the polychromatophilic (B) variety Later as the reticulocyte peak had passed and the

TABLE 10—*S ri l Eryth ocyte Protoporphyrin and Serum Iron Determ nations in Case 7 (B S)*

D te	Day f obser tio	Eryth ocyt p t porphyrin (EI)	Serum iron	Rem k
		g mma per 100 cc pack d R.B C	*g mma c*	
2/21/47	1		50	Period of crisis
2/23/47	2	8- 8		
2/25/47	5	83 3		
2/28/47	8		80	Postcrisis
3/ 3/47	11		80	
3/ 4/47	12	292 0		
3/13/47	21	137 6	50	
3/24/47	32	109 2		
4/14/47	53	65 0		Splenectomy April 16
4/17/47	56		90	
4/19/47	58	47 3		
4/21/47	60		10	
4/26/47	65	44 0		
4/28/47	67			
4/29/47	68	25 0		
5/ 1/47	70		30	
5/ 3/47	72	78 5		
5/ 5/47	74		50	
6/23/47	123		225	
9/ 5/47	197	30 0	150	

marrow picture was that as ordinarily seen in congenital hemolytic jaundice most of the normoblasts (table 9) were of the mature variety Thus the reticulocytopenia of the crisis could be explained adequately by a maturation arrest of the nucleated red cells of the marrow at the erythrogone level with a resultant lack in the production of mature non nucleated erythrocytes *

The large primitive red cells seen in the crisis have been referred to by some observers[17] as megaloblasts The concept of what a megaloblast is h been confused in the l terature in our laboratory this term is used for the nucleated red cell series as seen typically in pernicious anemia and related states The nucleus of the megaloblastic cell retains a relatively primitive chromatin mesh (which recalls that of the reticulum cell) until just before pyknosis The identity of the megalobl stic series has been discussed by Jones[22] Our studies indicate that the preponderant nucle ted r d cell—seen in the bone marrow during crisis—is not a megaloblast but is a pr m ti e red cell of the erythrogone (pronormoblast) type What

The maturation arrest of the nucleated red cells in the bone marrow is apparently not paralleled by an arrested maturation of the granulocytes which indeed appear to be increased in number, both in an absolute as well as a relative fashion Furthermore the relative proportions of the different types of granulocytes in the marrow are within normal limits suggesting that a maturation defect of these cells is lacking *These findings in the marrow in association with the leukopenia* and granulocytopenia of the peripheral blood might indicate that a block phenomenon is present i e although maturation of the granulocytes is proceeding in a normal fashion the mechanism controlling their delivery from the bone marrow to the blood is perhaps defective

Such a phenomenon occurs typically in various types of splenomegaly in which a possibly exaggerated activity of the spleen i e hypersplenism [10] is present

TABLE 11 —*Comparison of Blood Counts during Crisis in the Seven Cases*

Case	R B C	W B C	Reticulocytes	Remarks
	millions/cu mm	*thousands/cu mm*	*%*	
I D C M Jr	1 25	3800	—	
II R M Jr	1 22	3000	0 4	
III M McN	1 7	5000	0 7	
IV Mary D S	1 53	6600	0 3	
V H C	2 40	3850	0 1	
VI P O N	3 24	3300	7 6	
VII B S	1 3	15500 30% granulocytes	0 0	Minor hemolytic crisis

This is present in such diverse conditions as portal hypertension (cirrhosis of the liver) chronic infectious splenomegaly including malaria syphilis tuberculosis rheumatoid arthritis and Boeck s sarcoid Gaucher s disease various primary neoplasms of the spleen and finally in many cases of idiopathic splenomegaly Leukopenia neutropenia and thrombocytopenia i e pancytopenia are present although the bone marrow itself is hyperplastic Occasional cases of hypersplenic hemolytic anemia show low reticulocyte counts with a well-defined maturation arrest of nucleated red cells at the erythrogone level In these cases and from an analogy with other instances of hypersplenism [10] we have come to the conclusion that the hemolytic crisis is based on a suddenly developing splenic disturbance This in turn may be of infectious origin No definite evidence of infection has been advanced as yet but the occurrence of successive cases in the same family is strong presumptive evidence of this possibility Doan[13, 14] contends that the splenic hemolytic mechanism may be readily unbalanced with the result that numerous minor crises may develop during an individual s lifespan This might develop for example in the presence of certain acute infectious states

little maturation occurs beyond this primitive stage is productive of a normoblastic and not of a megaloblastic type of erythropoiesis

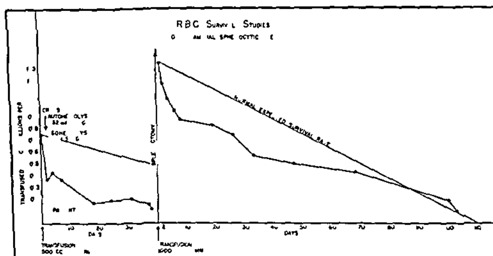


FIG 8a CASE 6 (P O N) Survival studies (Ashby technic) during crisis and after splenectomy The red cell survival time was diminished during crisis and became normal after splenectomy

FIG 8b CASE 7 (B S) The rate of disappearance of the transfused red blood cells pursues a straight line course i e normal survival time

It is possible that the postulated splenic abnormality leads (a) to an increase in hemolytic activity with resultant extreme spherocytosis and rapidly progressive anemia and (b) to various inhibitory hypersplenic effects Such effects upon the

bone marrow might result in (a) a block phenomenon in which granulocytes are prevented from being delivered normally to the circulating blood, (b) a reduced production of platelets from megakaryocytes and (c) in maturation arrest of red cells at the erythrogone (pronormoblast) level These phenomena would lead to reticulocytopenia leukopenia granulocytopenia and thrombocytopenia Thus the various features of the hemolytic crisis may be conceived of as being due to an extreme degree of hypersplenism causing simultaneously both excessive

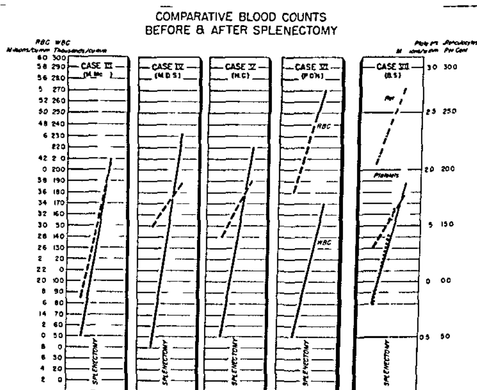


Fig 9 Comparative blood counts before and after splenectomy in cases 3–6 7 The darkened portions represent the period immediately preceding splenectomy In the white columns are the values obtained immediately following splenectomy Reticulocyte and platelet determinations are included only in case 7

hemolysis and maturation arrest of the nucleated red cells This would naturally lead to an extremely rapid development of anemia (figure 10)

The exact mechanisms by which blood becomes destroyed during the hemolytic crisis remain quite obscure There is no evidence of intravascular hemolysis as indicated by hemoglobinemia and hemoglobinuria The finding of abnormal iso antibodies in two of our cases (autohemolysin in case 6 autoagglutinin in small concentration in case 7) suggests an immune body type of autohemolytic activity as described in previous papers

This is confirmed by an abnormal survival time of transfused red cells in our case 6 No evidence of erythrophagocytosis is evident in the studies of splenic

histology The chief histologic feature of the removed spleen is the great pooling of blood within the pulp which shows large numbers of ghost cells and cells in various stages of hemolysis with the sinusoids appearing to be almost empty It would appear that the blood has largely left the sinusoids for the pulp where it is trapped It is possible that an infection may so alter the capillary permeability of sinusoids that blood leaks from them into the pulp where various mechanisms

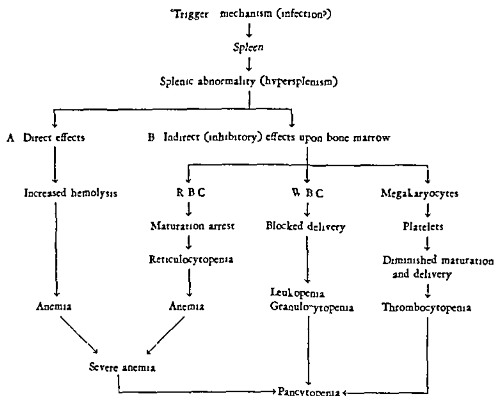


Fig 10 —The Hemolytic Crisis

such as erythrostasis mechanical trauma and abnormal antibody activity might combine in the destruction of excessive numbers of red cells

We do not believe as Owren[38] maintains that the events in the hemolytic crisis can be explained on the basis of a suddenly developing marrow aplasia In the first place the marrow is by no means aplastic Secondly the marrow is evidently capable of an extremely rapid increase in activity when the spleen has been removed This is borne out by the sudden rise in red cells white cells and platelets which occurs after splenectomy This is due we believe to the removal of a highly deleterious organ having inhibitory and phagocytic effects Furthermore Owren s concept does not explain the extreme degree of spherocytosis which regularly occurs during crisis and which indicates according to our investigations

bone marrow might result in (a) a "block" phenomenon in which granulocytes are prevented from being delivered normally to the circulating blood, (b) a reduced production of platelets from megakaryocytes and (c) in maturation arrest of red cells at the erythrogone (pronormoblast) level These phenomena would lead to reticulocytopenia leukopenia granulocytopenia, and thrombocytopenia Thus the various features of the hemolytic crisis may be conceived of as being due to an extreme degree of hypersplenism causing simultaneously both excessive

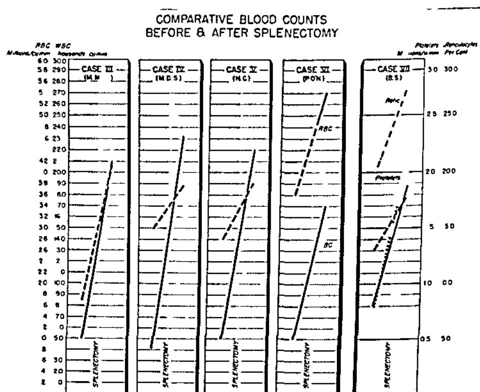


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represent an exaggeration of the normal hemolytic mechanism and since spherocytosis is so marked in the crisis we believe that the spherocyte does not necessarily indicate an inherent defect of erythrocyte formation but rather the end result of an abnormal type of hemolytic mechanism

That abnormal iso-antibodies are not found customarily in familial spherocytosis does not rule out their presence These antibodies may be of such minor intensity or adsorbed in some fashion to the red cell surface as to make their detection difficult by methods now available The failure to find abnormal antibodies need not necessarily indicate that they are lacking since methods for their detection may still be imperfect In the past two years alone two new methods have been introduced the use of bovine albumin as a diluting medium and the Coombs antiglobulin test These tests have resulted in the frequent finding of hemolytic antibodies undetected by methods previously in use They suggest that with the years other methods even more sensitive may become available

Similar reasoning holds true for the normal life span of red cells introduced into the circulation of an individual with the disease The fact that the foreign red cells are not destroyed more rapidly than normal has led to the widespread conception that the disorder must therefore be due to an inherent defect of the individual s own red cells This need not necessarily be true since the abnormal hemolysis may be autospecific (i e active only against the individual s own red cells) Furthermore our finding in one case of crisis of a greatly diminished red cell survival time (see figure 8*a* case 6) might indicate that in crisis the hemolysin activity may become so outspoken that it will not only cause a rapid destruction of the individual s own cells but also result in an exponential type of destruction of *introduced foreign* cells

Summary

The course of hereditary spherocytosis (congenital or familial hemolytic anemia) is subject to major or minor exacerbations or crises Pancytopenia reticulocytopenia and extreme spherocytosis characterize the major crises during which hypersplenic effects appear to play a major role These are characterized by the combination of (1) an unusual degree of hemolysis with (2) inhibitory effects upon maturation and delivery of bone marrow cells At the height of the crisis an extreme degree of maturation arrest in erythropoiesis is present Splenectomy which is often urgently necessary results in a very rapid increase in all the cellular elements of the blood confirming the phagocytic and inhibitory effects of the abnormal spleen Following splenectomy no further crises occur The presence of successive cases in the same family suggest the possible role of infection as a precipitating or trigger agent in initiating an abnormal splenic mechanism leading to crisis The cause of the hereditary spherocytosis is commented upon and evidence bearing upon an autopsecific mechanism is discussed

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[1] Brown C M Hayward O C Powell E O and Witts L J De truction of transfused erythrocytes in anemia J Path Bact 56 81 1944

a marked degree of hemolytic activity Studies of the fecal urobilinogen should settle this problem These were not carried out by Owren nor in our own series except in case 7 where it was distinctly elevated Dr Jonah Li (University of Oregon Medical School) recently studied a family of five individuals having hemolytic crises occurring within ten days of each other All the cases showed blood pictures similar to those described above with extremely low reticulocyte values In two cases fecal urobilinogen determina ions at the height of the crises showed values that averaged approximately 1500 mgs per day i e about 20 times the normal value taking into account the severe anemia At the termination of the crises the fecal urobilinogen values became very low

A final indication of the direct relationship of the spleen to the crisis is the com plete disappearance of all tendency to crisis following splenectomy Whereas be fore operation the red cell count is subject to well defined and even to marked fluctuations this does not occur following operation Spherocytosis continues to be present but the sudden extremes in this abnormality in association with a greatly increased anemia do not develop This is another indication that the spleen has an active influence in blood destruction and in initiating the various events of the hemolytic crisis

The Possible Relation of the Events in the Hemolytic Crisis to the Normal Hemolytic Activity

The sudden development of extreme spherocytosis and extreme red cell destruc tion in the crisis suggests strongly an extrinsic activity i e a factor outside the marrow resulting in changes affecting the non nucleated red cell The theory most commonly held for the spherocytosis of the familial disease is that it is the product of an abnormal type of erythropoiesis Since spherocytosis is most marked in the crisis one would expect to find some evidence of this abnormality in the bone marrow However this is by no means the case either in the chronic form of the disease or during the crisis In the chronic form the marrow shows marked ery throblastic hyperplasia maturation is orderly and a gradual reduction in size of the nucleated red cells occurs as the mature orthochromatic stage is reached No evidence for the development of spherocytosis can be seen in the first stages of the life span of the non nucleated erythrocyte (i e the polychromatophilic red cell) in which the cells show a diameter greater than normal The discrepancy between the relatively large polychromatophilic reticulocytes and the small orthochromatic spherocytes is quite striking suggesting that some abnormal mechanism modifies the rather young circulating erythrocytes making them spherocytes This is noted even more strikingly in acute acquired hemolytic anemia with abnormal iso antibodies and can be reproduced experimentally

In the extreme degrees of spherocytosis as seen in the hemolytic crisis one would expect if the theory of a bone marrow abnormality were correct to find an extreme degree of spherocytic development in the marrow Instead the marrow shows very large primitive cells with an almost complete maturation arrest We believe that this can only indicate that the spherocytes become spherocytic outside the marrow perhaps by some sort of abnormal intravascular mechanism Since the crisis may

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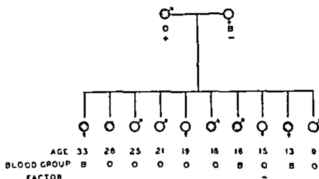


Fig 1 *Family Ca*

TABLE 1 —*Blood Find gs in the Ca Family*

	M C	Mrs C	C	J	J	T	Ph	Ay	Fr	M	An	Ro
Ag			33	28	25	21	19	18	16	15	13	9
Sex	M	F	F	M	M	M	F	M	M	F	F	M
Bl od m al												
R B C M per cu mm	5 6	4 8	5 5	6 0	4 9	4 5	4 1*	5 8	5 8	4 4*	5 6	4 4
Hb %	75	76	71	81	90	92	79	75	66	84	66	82
Hb Gm per 100 cc blood	11 7	11 8	11 1	12 6	14 0	14 4	12 3	11 7	—	13 1	10 3	12 8
Hematocrit %	40 3	39 1	38 0	39 8	43 3	42 6	38 6	35 2	35 4	40 2	38 2	40 1
M C V cu μ	72 2	81 1	69 8	66 8	88 7	94 6	94 4	60 9	60 8	91 8	68 4	90 7
M C H concentr %	29 0	30 3	29 0	31 8	32 4	33 7	31 9	33 2	29 1	32 6	27 0	31 9
M C H micromicrograms	21 0	24 6	20 4	21 2	28 8	31 9	30 1	20 2	17 7	29 9	18 4	28 9
Reticulocytes	1 1	6	3 1	1 0	0 5	0 8	1 3	0 8	2 6	0 8	1 0	1 5
Nucleated red cells	0	0	0	0	0	0	0	0	0	0	0	0
Stippling	0	0	0	+	0	0	0	+	+	0	0	0
Icteric index	—	5	—	—	5	7	—	—	4	5	5	—
W B C thousands per cu mm	8 2	7 8	6 8	7 9	6 5	5 3	11 5	8 3	10 3	7 4	6 8	5 6
Blood group	O	B	B	O	O	O	O	O	B	O	B	O
Blood type	N	N	N	N	N	N	N	N	N	N	N	N
Rh factor	+	−	+	+	−	+	−	+	−	−	−	+
Blood smear	Abn	N	Abn	Abn	N	N	Abn	Abn	Abn	Abn	Abn	N

Counts taken following satisfactory therapy with iron
N norm l Abn abnormal
Red Cell Resistance—Per cent hemolysis in hypotonic solutions of sodium chloride

	1	10^m	50^m	75^m
Francis	42	37	32	29
Normal	43	38	35	33

under the itle Benign Familial Polycythemi b t since it is no v believed that this condition is identical to Cooley s trait the essential d ta are presented in table 3 and figure 3

Family Pa The oldest da ghter of this f mily was first obser ed at the Massachusetts General Hospi tal in 1934 when she was 19 years of age At this time she h d acute pyelonephritis and in addition was

15

THE GENETIC RELATION AND CLINICAL DIFFERENTIATION OF COOLEY'S ANEMIA AND COOLEY'S TRAIT

By Geneva A Daland B S and Maurice B Strauss M D

IN 1925[1] the late Thomas B Cooley first described the severe and generally fatal anemia that is now variously known as Cooley's Anemia Mediterranean anemia and thalassemia Since then numerous workers[1–10] have reported cases of a mild blood disorder which resembles Cooley's anemia but is not associated with symptoms or disability This disorder found almost exclusively in Italian and Greek families is characterized by microcytosis and hypochromia with marked variation in the size and shape of the erythrocytes and by increased red cell resistance to lysis in hypotonic salt solution The hemoglobin values are either normal or moderately reduced while the red blood cells are generally increased in number For reasons to be discussed later the term Cooley's Trait is proposed for this disorder and will be used throughout this paper Neel and Valentine[11] have estimated that this condition is present in 4 per cent of persons of Italian ancestry in Rochester N Y and one of us has noted it frequently as an incidental finding in soldiers of Mediterranean ancestry in an Army General Hospital

Since earlier publication [5] 4 additional families have been studied one family including 3 cases of Cooley's anemia One of the patients with Cooley's anemia has given birth to a living child an event thus far unreported This child exhibits Cooley's trait

It is the purpose of this publication to present the hematologic and genetic data obtained from the examination of these families to clarify further the inheritance of both Cooley's trait and Cooley's anemia and to emphasize the clinical and hematologic differentiation of the two conditions

Observations

Family Ca A 16 year old boy of Italian parentage came under observation during an acute attack of rheumatic fever He was found to have a hypochromic microcytic anemia with marked variation in red cell size and shape and increased red cell resistance to lysis by hypotonic salt solution Twelve grains of ferrous sulfate daily for one month failed to alter his blood values Blood studies on the patient on the patient's father mother and 9 siblings are recorded in table 1 and figure 1 All the members of the family were healthy and completely asymptomatic No member was known to have been anemic or seriously ill

Family Cr A 32 year old woman first came under observation after she had been unsuccessfully treated for a mild anemia for several years Her blood study exhibited all the characteristics of Cooley's trait Accordingly other members of the family were examined The data are shown in table 2 and figure 2 Both parents had been born near Naples Italy The father had died many years before The mother died of pneumonia in 1941 In 1938 she had been observed to have undue variation in the size and shape of her erythrocytes and considerable basophilic stippling When she had pneumonia she was anemic and nucleated red blood cells were observed in stained blood films

Family Sp This family was reported in detail from this laboratory by Spodaro and Forkner[3] in 1933

From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) Boston City Hospital the Medical Service Cushing Veterans Administration Hospital and The Department of Medicine Harvard Medical School

TABLE 2 — *Blood Findings in the Cr Family*

	Mrs Cr	Children							C and F ll e		
		Mi	Lo	M T	L D	J C	P	An I	F T	S D	A H
Age	50	35	34	32	30	28	27	25	4	2	4 days
Sex	F	M	F	F	F	F	M	M	M	F	M
Blood examination											
R B C M per cu mm	4.7	5.7	4.6	4.8	5.4	4.4	5.7	6.4	6.2	5.3	6.3
Hb %	95	100	87	69	87	66	101	83	84	92	123
Hb Gm per 100 cc blood	14.8	15.6	13.5	10.7	13.6	10.3	15.7	12.9	13.1	14.3	19.2
Hematocrit %	42.4	46.0	38.7	32.3	40.0	30.3	46.4	39.7	39.4	—	—
M C V cu μ	90.2	82.1	84.1	67.5	75.4	70.4	81.4	62.0	63.5	—	—
M C H concentr %	34.9	33.9	34.0	33.3	33.9	33.9	33.5	32.6	33.2	—	—
M C H micromicrograms	31.5	27.8	29.5	22.4	35.6	23.9	27.6	20.2	21.1	—	—
Reticulocytes %	—	2.6	1.7	2.9	1.3	3.4	1.2	1.4	2.3	—	—
Nucleated red cells	0	0	0	+	0	+	0	0	+	0	0
Stippling	+++	+	0	+	0	+	0	+	+	0	0
Icteric Index	—	6	5	4	5	5	5	7.5	6	—	—
Red cell resistance											
Trace of hemolysis	47	46	46	42	46	—	46	44	42	—	—
Complete hemolysis	32	29	29	17	29	—	27	18	17	—	—
Platelets thousands per cu mm	—	270	286	244	300	229	286	174	300	—	244
White blood cells thousands per cu mm	9.8	6.6	6.0	8.0	9.0	10.5	6.9	5.0	10.0	9.1	9.5
Polymorphonuclear neutrophils %	55.0	44.0	55.0	72.0	55.0	70.0	44.0	45.0	50.5	25.0	46.5
Polymorphonuclear eosinophils %	7.0	2.0	1.0	2.0	3.0	3.0	3.0	2.0	3.0	0	6.5
Polymorphonuclear basophils %	0	0	1.0	1.0	3.0	1.0	1.0	0	0.5	0	0.5
Small lymphocytes %	30.0	45.0	39.0	21.0	36.0	20.0	45.0	17.0	38.0	70.0	8.0
Large lymphocytes %	0	0	0	0	0	0	0	34.0	0	0	35.5
Monocytes %	8.0	9.0	4.0	4.0	3.0	6.0	7.0	2.0	8.0	5.0	3.0
Abnormal or young cells %	0	0	0	0	0	0	0	+	0	0	0
Myelocytes %	0	0	0	0	0	0	0	0	0	0	0
Blood smear	Abn	N	N	Abn	N	Abn	N	Abn	Abn	N	N

N normal Abn abnormal

discovered to have the characteristic blood picture of Cooley s anemia as well as jaundice splenomegaly and hanges in the bones She maintained approximately the same blood level for the next five years but died with severe anemia a fortnight after the birth of a child At the time of delivery her red blood count was reported to be 1 12 million per cu mm and the hemoglobin 3 9 Gm per 100 cc

In 1934 when the diagnosis of Cooley s anemia was first made on the above patient her parents and 5 siblings were examined At that time a brother aged 7 and the youngest sister aged 16 were considered to have Cooley s anemia but the mother father and 3 other sisters were believed to be normal On subsequent examination by the authors however all but one sister were found to have Cooley s trait (table 4 fig 4) The brother had many hospital admissions and finally died at the age of 11 Necropsy findings were typical of Cooley s anemia

The youngest sister has been followed in the Thorndike Memorial Laboratory since 1938 during which time she has continued to present a characteristic picture of Cooley s anemia In March 1946 she was delivered of a male infant whose blood has been examined on several occasions On October 1 1946 his red cells numbered 5 330 000 per cu mm and his hemoglobin was 9 4 Gm per 100 cc Following treatment with iron for two months the red cell count was 6 100 000 and the hemoglobin 14 0 Gm His blood films are definitely abnormal showing variation in size of the red cells variation in intensity

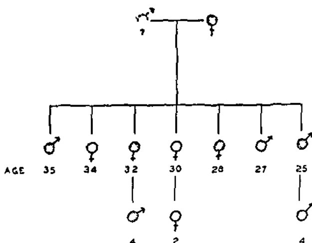


Fig 2 *Family Cr*

of staining microcytes occasional pencil forms and fragmented forms No stippled cells were seen This picture has been maintained throughout the observations and is a distinctly unusual picture when the red count and hemoglobin are so high With the exception of frequent colds he has been entirely well His father has an entirely normal blood (table 4)

Literature

During 1940 and 1941 three groups of investigators independently described the mild blood disorder illustrated by the cases presented above Wintrobe and his co workers[3] considered the condition to be a benign form of Cooley s anemia Dameshek[4] described it as target cell anemia These authors both then and in later publications [9] [14] emphasized the existence of a continuous series of conditions from the very mild cases of Cooley s trait to the most severe Cooley s anemia The present authors[5] termed the mild disorder Familial Microcytic Anemia stressing the fact that although it resembled Cooley s anemia clinical and hematologic differentiation was not difficult It was further stated that the mild disorder

anemia frequently exhibited increased red cell resistance to lysis by hypotonic salt solution it was not until Wintrobe[14] pointed out that in two different patients with Cooley's anemia *both* parents exhibited the mild blood disorder that the genetic relationship between the two conditions became apparent Wintrobe's observations were confirmed by Smith[7, 8] Dameshek[9] and Valentine and Neel[10] who in 1944 published a thorough discussion of the subject and added data from four additional families

Differentiation of Cooley's Trait from Cooley's Anemia

Although it is possible by proper selection of cases to show a continuous series of conditions grading from the most severe type of Cooley's anemia fatal in early life to the most mild instances of Cooley's trait in which there is no anemia whatsoever it is our impression that generally the differentiation of the two conditions is not difficult although both are characterized by hypochromia microcytosis

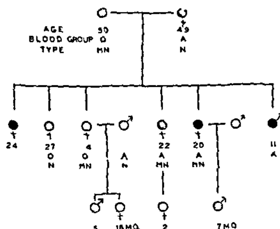


FIG 4 *Family Pa*

anisocytosis and poikilocytosis out of proportion to the hemoglobin levels The blood pictures fall into two fairly well defined groups one severe and generally fatal the other benign Correlated with these two blood pictures may be found marked clinical signs and symptoms in the case of Cooley's anemia or their absence or minor nature in Cooley's trait The differential diagnostic findings between Cooley's anemia and Cooley's trait are shown in table 5

Genetic Considerations

Three main types of inheritance could explain the relationship between Cooley's anemia and Cooley's trait[10]

1 *A single dominant factor variably expressed* An example of this type of inheritance is found in the variable severity of hemophilia which the sons of one mother may exhibit each of whom owes his disease to an identical gene If this mode of inheritance were involved Cooley's anemia should appear in families

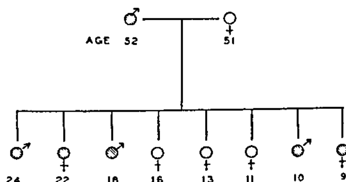


Fig 3 *Family Sp*

Table 3 —*Blood Findings in* Sp *Family* (*Ref* 2)

Age	52	51	24	22	18	16	13	11	10	9
ex	M	F	M	F	M	F	F	F	M	F
Bl d m at o										
R B C M per cu mm	6 8	5 5	8 0	7 1	8 1	5 0	5 6	4 9	6 4	5 2
Hb Gm per 100 cc blood	13 6	14 6	15 8	12 7	14 5	12 3	15 4	12 5	12 5	12 8
Hematocrit %	50 3	46 7	50 0	49 5	57 0	—	46 9	45 2	42 6	39 6
M C V cu μ	74 2	85 6	63 2	69 7	70 6	—	83 5	92 6	66 2	76 2
M C H concentr %	27 0	31 2	31 5	25 7	25 5	—	32 8	27 6	29 3	32 3
M C H micromicrograms	20 0	26 -	19 7	17 9	18 0	—	27 3	25 6	19 4	24 6
Reticulocytes %	2 4	1 6	—	2 2	1 0	0 6	1 2	1 4	2 4	0 2
W B C thousands per cu mm	8 1	6 9	8 5	7 0	8 8	7 3	11 4	8 3	8 0	8 3
Polymorphonuclear neutrophils %	63 0	66 0	63 0	63 0	57 0	58 0	56 0	64 0	64 0	50 0
Polymorphonuclear eosinophils %	2 0	1 0	—	2 0	4 0	1 0	2 0	1 0	3 0	4 0
Polymorphonuclear basophils %	—	1 0	4 0	1 0	2 0	3 0	3 0	2 0	1 0	1 0
Small lymphocytes %	28 0	28 0	21 0	31 0	21 0	31 0	31 0	25 0	22 0	41 0
Monocytes %	7 0	4 0	12 0	3 0	16 0	7 0	8 0	8 0	10 0	4 0
Platelets	Normal	Normal	+	+	Normal	Normal	+	−	+	Normal

appeared to be inherited as a dominant character whereas Cooley s anemia was believed to be recessive [15, 16] Although Angelini [17] and independently Caminopetros [15] had observed that the parents and siblings of patients with Cooley s

anemia frequently exhibited increased red cell resistance to lysis by hypotonic salt solution it was not until Wintrobe[14] pointed out that in two different patients with Cooley s anemia *both* parents exhibited the mild blood disorder that the genetic relationship between the two conditions became apparent Wintrobe s observations were confirmed by Smith [7] [8] Dameshek[9] and Valentine and Neel [10] who in 1944 published a thorough discussion of the subject and added data from four additional families

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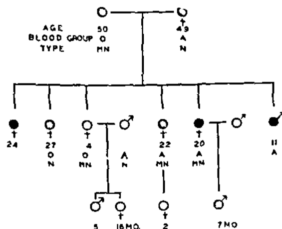


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in which only one parent exhibited a blood abnormality However, in essentially every case in which both parents of a patient with Cooley s anemia have been examined both have shown the mild disorder Since, in accord with this theory only one parent need be affected and since marriages between two affected in dividuals should be much rarer than between one affected and one normal person

TABLE 4 —*Blood Findings in the* Pa *Family*

	Mr P		Mr P		M P S		J P B	
Age	50		49		24		26	
Se	M		F		F		F	
Blood m tion								
R B C M per cu mm	5 6		6 0		3 6		5 0	
Hb %	86		92		52		84	
Hb Gm per 100 cc blood	13 4		14 3		8 1		13 1	
Hematocrit %	43 9		44 5		27 4		41 1	
M C V cu μ	77 3		74 4		76 1		82 8	
M C H concentr %	30 6		32 2		29 6		32 0	
M C H micromicrograms	23 6		24 0		22 5		26 5	
Reticulocytes %	0 6		0 7		11 8		0 /	
Nucleated red cells	0		0		+		-|	
Stippling	++		+		+		+	
Icteric index	4		4		—		3	
Red cell resistance*								
			% H m	NaCl	H m	NaCl	% Hem	NaCl
			1	49	Trace	58	1	46
			10	41	Complete	16	10	40
			50	37			50	34
			75	34			75	32
Platelets thousands per cu mm	+		N		+		N	
White blood cells thousands per cu mm	5 9		8 8		1- 4		6 8	
Polymorphonuclear neutrophils %	57 0		49		55		48	
Polymorphonuclear eosinophils %	3		3		1		9	
Polymorphonuclear basophils %	—		—		—		2	
Small lymphocytes %	25		27		18		28	
Large lymphocytes %	9		11		19		5	
Monocytes %	4		8		5		5	
Abnormal or young cells %	1		1		—		3	
Myelocytes %	1		1		2		—	
Blood group	O		A		—		O	
Blood type	MN		N		—		N	
Rh factor	+		+		—		+	
Blood Smear	Abn		Abn		Abn		Abn	

TABLE 4 —*Continued*

	J P C	A P C	A P T	M P
Age	23	22	20	11
Sex	F	F	F	M
Blood exam at				
R B C M per cu mm	4 8	5 0	5 1	2 5
Hb %	105	76	62	28
Hb Gm per 100 cc blood	16 4	11 8	9 7	4 4
Hematocrit %	44 6	40 6	29 0	
M C V cu μ	92 3	80 2	56 9	
M C H concentr %	36 7	29 2	30 0	
M C H micromicrograms	33 9	23 4	19 0	
Reticulocytes %	0 8	1 5	2 8	6 2
Nucleated red cells	0	0	+	+++
Stippling	0	++	++	++
Icteric index	4	5	10	
Red cell resistance*	% Hem % NaCl 1 46 10 41 50 37 75 35	% Hem % NaCl 1 44 10 39 50 35 75 33	% Hem % NaCl 1 49 10 43 50 28 75 23	
Platelets thousands per cu mm	N	N	264	+
White blood cells thousands per cu mm	11 8	8 6	11	4 4
Polymorphonuclear neutrophils %	60	72	70	56
Polymorphonuclear eosinophils %	2	2	1	—
Polymorphonuclear basophils %	1	—	—	—
Small lymphocytes %	20	17	14	25
Large lymphocytes %	10	—	6	—
Monocytes %	7	9	7	15
Abnormal or young cells %	—	—	1	4
Myelocytes %	—	—	1	—
Blood group	O	A	A	A
Blood type	MN	MN	MN	—
Rh factor	+	+	+	—
Blood Smear	N	Abn	Abn	Abn

* Red cell resistance is expressed as percentage of cells hemolyzed in a given concentration of sodium chloride solution

Daland and Worthley[12] normal value Trace of hemolysis 48 NaCl Complete hemolysis 26 NaCl

Ham and Shen[3] normal value 1% hemolysis 43 10% hemolysis 38 50% hemolysis 35 75% hemolysis 33 NaCl

N normal Abn abnormal

TABLE 4 —Concluded

	Family of J P C			Family of A P C	Family of A P T	
	Mr C	P C	K C	J C	Mr T	D T
Age	33	5 yr	16 months	2	—	7 months
Sex	M	M	F	F	M	M
Blood examination						
R B C M per cu mm	5 5	4 8	5 0	—	5 6	5 3
Hb %	108	85	86	85	116	60
Hb Gm per 100 cc blood	16 9	13 2	13 4	—	18 0	9 3
Hematocrit %	49 5	38 2	—	—	50	—
M C V cu μ	90 0	80 2	—	—	90 0	—
M C H concentr %	34 1	34 6	—	—	35 8	—
M C H micromicrograms	30 7	27 7	26 9	—	3 -	17 6
Reticulocytes %	1 6	1 1	0 6	—	—	—
Nucleated red cells	0	0	0	—	—	0
Stippling	0	0	0	—	—	0
Icteric index	5	5	—	—	7	—
Platelets	N	N	N	—	N	N
W B C *thousands per cu mm*	6 7	9 7	—	—	8 7	9 3
Blood Group	A	O				
Blood Type	N	MN				
Rh factor	—	+				
Blood Smear	N	N	N	N	N	Abn

K C and J C show a few young cells which are to be expected at their ages R B C are normal D T showed more variation in size and shape than normal Cells were somewhat hypochromic

TABLE 5 —*Clinical and Hematologic Differentiation of Cooley s Anemia and Cooley s Trait*

	Cooley s Anemia	Cooley s Trait
Red Blood Cell Count	<5 000 000	5 000 000–8 000 000
Mean Corpuscular Volume	50 to 100 cu micra	55 to 80 cu micra
Hemoglobin	<10 Gm per 100 cc	9 to 16 Gm per 100 cc
Nucleated Red Blood Cells	Common	Rare
Reticulocytes	Increased	<4 per cent
Polychromatophilic Cells	Increased	Occasional
Stippled Cells	Marked	Frequent
Icteric Index	Increased	Normal
Leukocytes	Increased	Normal
Poikilocytosis	Extreme	Moderate
Bone Changes	Generally in skull and other bones	Skull only (slight)
Splenomegaly	Common	Occasional
Features	Mongoloid	Normal
Age	Young	Any
Prognosis	Poor	Excellent

most cases of Cooley s anemia would be the issue of the latter type of union

2 *The simultaneous presence of two nonallelomorphic dominant factors one inherited from each parent* as suggested by McIntosh and Wood [18] would result in Cooley s

anemia This theory requires the assumption that each factor alone leads to the same mild somatic condition which Valentine and Neel[10] consider improbable However it may be noted that in drosophila a number of different factors may lead to the identical eye color There are other more complex types of inheritance which may be considered in which two nonallelomorphic factors are required for the development of the full blown character and in which heterozygosity leads to a partial abnormality For example ski wing in drosophila results from homozygosity of two genes in the second and third chromosomes If the gene in the second chromosome is heterozygous and the gene in the third chromosome homozygous a mild form of ski wing results

3 *A single incomplete recessive or dominant factor* Under this type of inheritance the heterozygote would exhibit Cooley s trait and the homozygote Cooley s anemia

The data presented here together with the extensive statistical analysis of Valentine and Neel[10, 19] are compatible with either the second or the third mode of inheritance

Nomenclature

Spodaro and Forkner[3] termed this mild blood disorder benign familial polycythemia Since polycythemia is not necessarily present this designation is unsuitable The present authors have called it familial microcytic anemia [5] a name which must be rejected since anemia may be absent

Wintrobe et al [2] called this condition a familial hemopoietic disorder in Italian adolescents and adults resembling Mediterranean disease (thalassemia) Dameshek[4] referred to it as target cell anemia However target cells are encountered in many conditions and may be artificially produced in vitro by suspending normal cells in plasma or serum rendered hypertonic [0] Later Dameshek used the designation familial Mediterranean target oval cell syndromes [9] Since cases have been reported in English Negroes Chinese Turkish Spanish and others [1] and since there is a distinct blood disorder characterized by oval erythrocytes this name does not appear suitable Valentine and Neel s[10] terminology thalassemia major and thalassemia minor for the severe and mild disorders has much in its favor However the wide distribution of these conditions even if their common origin at one time was the Mediterranean and the cumbersome terms have led the present authors to call the conditions Cooley s anemia and Cooley s trait bearing in mind the analogous sickle cell anemia and sickle cell trait It is not implied that sickle cell anemia and sickle cell trait bear the same genetic relation as Cooley s anemia and Cooley s trait Although eponymic nomenclature is in general undesirable it may be pointed out that a number of syndromes such as Graves disease which may occur without goiter without thyrotoxicosis without ophthalmopathy defy accurate descriptive terminology

Summary

1 Four additional families illustrating the clinical and genetic relationships of Cooley s anemia and Cooley s trait have been presented

2 Blood findings in an offspring of a patient with Cooley s anemia are recorded

3 The asymptomatic nature of Cooley s trait and its differentiation from Cooley s anemia has been emphasized

4 The inheritance of Cooley s trait and Cooley s anemia may be best explained in terms of an incomplete dominant or of the simultaneous appearance of two nonallelomorphic genes

ACKNOWLEDGMENT

We are indebted to Dr John H Linner for many of the observations on the Ca family and to Miss Clara Gillette for observations on the Cr family

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STUDY OF THALASSEMIA MINOR IN THREE GENERATIONS OF AN ITALIAN FAMILY

By ROBERT W HEINLE M D AND MARGARET RUTH READ M D

A CONSIDERABLE knowledge concerning the heredity and transmission of Cooley s erythroblastic anemia or thalassemia has accumulated during the past twenty years but there is still need for more complete data and further genetic studies of families showing the trait

In 1927 Cooley[1] reported his observations on erythroblastic anemia occurring in Mediterranean peoples He concluded that the anemia was congenital but in spite of frequent familial occurrence he doubted that heredity was a factor since the patients died before puberty and so could not transmit the disease

It was recognized for the first time in 1937 that a mild but similar type of anemia occurred in parents and siblings of individuals with Cooley s anemia Angelini (quoted by Wintrobe and his associates[2]) observed that in some instances the erythrocytes of apparently healthy parents and siblings of patients with Cooley s anemia showed decreased fragility when tested in hypotonic saline Caminopetros[3] independently confirmed this observation in 1938

Two years later Wintrobe and co-workers Dameshek[4] and Strauss and co-workers[5] described a mild form of microcytic hypochromic anemia resistant to iron therapy and occurring in Italian families The anemia was characterized by the frequent occurrence of increased numbers of erythrocytes with absolute and relative reduction of hemoglobin bizarre forms of erythrocytes and decreased fragility in hypotonic saline These authors variously termed the dyscrasia as target cell anemia familial microcytic anemia and anemia of adults resembling thalassemia It was not immediately apparent whether this disease was related genetically to Cooley s Mediterranean anemia but such a possibility was suggested

Wintrobe[6] later confirmed Angelini s and Caminopetros observation of decreased fragility of erythrocytes in the parents of patients having Cooley s erythroblastic anemia and further pointed out that the blood picture in the parents was identical with the familial microcytic hypochromic anemia which he had previously described [2]

In 1942 and 1943 Dameshek[7] described several anemic states of varying degree of severity ranging from Cooley s erythroblastic anemia to conditions with mild hypochromic anemia target oval and stippled cells and increased resistance of the erythrocyte to hypotonic saline He confirmed the findings of other workers in demonstrating that such blood changes occurred in both siblings and parents of patients with thalassemia Smith[8] also demonstrated similar changes in the blood of siblings of patients with Cooley s anemia and discussed the diagnosis of the trait or mild form of the disease

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Valentine and Neel [9] in reporting studies on parents and siblings of 3 patients with thalassemia and of one person with a similar mild condition considered the hereditary aspects of the disorder and emphasized the problem of differential diagnosis and the clinical significance of the mild form of the anemia By statistical analysis of cases collected from the literature they concluded that the bulk of evidence favored the hypothesis that the mild microcytic hypochromic anemia was a form of thalassemia which resulted from heterozygosity for a factor which when homozygous caused full blown thalassemia These authors suggested the term thalassemia major for the severe erythroblastic anemias as originally described by Cooley and thalassemia minor for the mild microcytic hypochromic anemias characterized by target cells, oval cells and increased resistance of the erythrocytes to hypotonic saline

Cooley [10] has more recently described a microcytic hypochromic anemia oc curring only in males with transmission through the females in a family of Dutch descent His conclusion that the disease was due to a fundamental constitutional defect of the hematopoietic system unrelated to familial microcytic anemia may be open to some doubt

In 1945 Neel and Valentine [11] reiterated their views regarding thalassemia major and minor and the inheritance of the conditions From a study of a portion of the Italian population of Rochester N Y they concluded that thalassemia major occurred in about 0 042 per cent and thalassemia minor in about 4 per cent

The present report deals with the occurrence of a microcytic hypochromic anemia in 9 of 13 members of a family of Sicilian descent over a span of three generations The study was initiated when one of the members of the second generation was found to have a hypochromic anemia which did not respond to iron

Case Report

Patient L O a 37 year old unmarried woman of Sicilian descent was first seen by us on Septemb r 18 1945 complaining of feeling faint She had first noted this two years previously and was told by a phy sician that she had anemia Therapy with iron liver extract injections and oral liver iron preparations were without symptomatic or hematologic benefit She later experienced fatigue and a sensation of twitching in the left leg and hand although no muscle contractions were ever visible She had been studied at another hospital on two occasions but was not aware that any diagnosis was made History of anemia in other members of her family could not be elicited

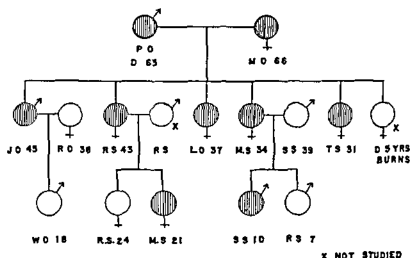
On physical examination th positive findings consisted of slight pallor of the mucous membranes a metallic first h art sound at th mitral area bilateral positive Hoffman reflex and a nystagmus on right lateral gaze with fast component to the right The liver and spleen were not palpable

She had b en examined by a neurologist who was unable to explain the symptoms and signs and who considered that they might be secondary to the anemia They have never been adequately explained

Laboratory data Erythrocytes 5 570 000 per cu mm hemoglobin 10 4 Gm per 100 cc leukocytes 5 300 hematocrit 36 mean corpuscular volume 64 9 cu microns mean corpuscular hemoglobin 18 7 micromicrograms mean corpuscular hemoglobin concentration 28 9 per cent Differential blood count 58 5 per cent neutrophils 3 5 per cent eosinophils 0 5 per cent basophils 31 0 per cent lymphocytes 6 5 per cent monocytes No nucleated red cells were seen The erythrocytes showed marked anisocytosis and poikilocytosis and were microcytic hypochromic Numerous target cells and a few oval shaped cells were present (figs 1 and 2) The platelets were slightly increa ed in number Sedimentation rate was slower than normal the corrected val e b ing less than zero This finding was due presumably to delay in rouleau formation resulting from the abnormal shape of the erythrocytes

Fig 1 Patient L O second generation Peripheral blood ×385

Fig Patient L O econd g ne at o Peripheral blood showing target cells oval cells poikilocytes and hypochromia ×640



FAMILIAL MICROCYTIC ANEMIA

FIG 3 Occurrence of thalassemia minor in three generations of a family of Italian origin Numbers after initials indicate age of patients in years

TABLE 1

Patient	Sex	Generation	Age	RBC × 10⁶	Hgb	WBC × 10	PCV	MCV	MCH	MCHC	Bizarre cells	Target cells	Ret	Spleen below costal margin	Icterus Index	Fragility Test % saline: Beg	Fragility Test % saline: Comp
					Gm			cu mic	micro-micro-gm	%			%	cm			
P O	M	1	63	4 02	11 0	5 550			26 1		+	+					
				3 90	10 4	7 110											
M O	F	1	66	5 50	12 0	5 900	40	72 7	21 9	30 0	+	+		0	8	0 46	0 30
L O	F	2	37	5 57	10 4	5 300	36	64 9	18 7	28 9	++++	++++		0		0 38	0 20†
R S	F	2	43	4 84	10 4	5 150	34 5	71 5	21 6	30 2	++	+		0 5	8	0 34	0 20
J O	M	2	45	6 33	12 7	8 500	45	71 0	20 1	28 2	++	++		2	2	0 42	0 20†
T S	F	2	31	6 53	11 9	10 900	41	62 7	18 2	29 1	+	+		1	8	0 38	0 10‡
M S	F	2	34	5 33	11 0	10 200	38	71 4	20 8	29 2	++++	++++	0 1	0	5	0 36	0 20†
M S	F	3	21	6 99	12 2	6 700	38	54 4	17 4	32 1	++++	+	0	0 5		0 42	0 22†
R S	F	3	24	4 90	13 4	7 950	42 5	86 7	27 2	31 5	0	0		0 5		0 44	0 30‡
R S	M	3	7	5 03	13 1	10 200	41	81 5	26 0	31 2	0	0	0 3	0	6	0 44	0 32‡
S S	M	3	10	6 42	11 5	7 300	40	62 3	17 8	28 /	+	++++	0 2	0	4	0 44	0 22†
W O	M	3	18	5 3	15 9	7 500	4/ 5	88 6	29 7	33 5	0	0		0		0 46	0 24‡
R O	F	S 2*	38	4 68	13 4	6 500	44	94 0	28 7	30 4	0	0		0			‡
S S	M	S –	39	5 36	15 6	6 500	46	86 8	29 1	31 7	0	0		0		0 44	0 32‡

S 2 = spouse of member of second generation
† Platelets estimated from smear increased in number
‡ Platelets estimated from smear normal in number

She was seen again one month later with the same complaints and laboratory findings There had been no response to iron therapy Saline fragility test showed beginning hemolysis at 0 38 per cent and

complete hemolysis at 0 20 per cent X rays of the skull humerus and hands showed a slight degree of demineralization without specific changes

It was felt that much of the patient s symptomatology was on a nonorganic basis especially since she was inclined to place the blame for her symptoms on her work (machine operator) and since it seemed likely that the anemia had antedated the onset of her symptoms

A tentative diagnosis of familial microcytic anemia was made and it was arranged to study other members of her family

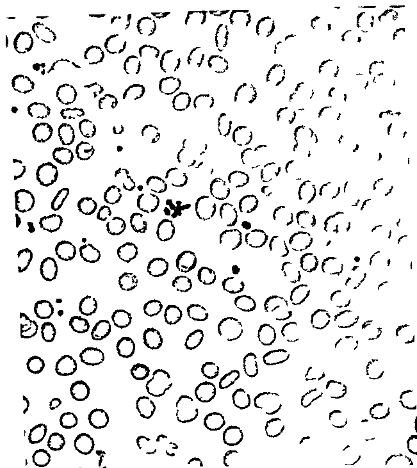
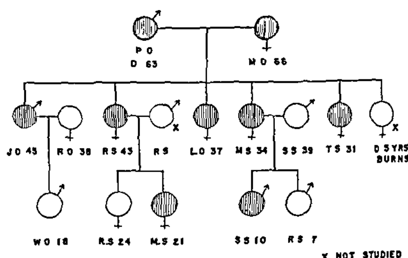


Fig 4 Patient S S third generation Peripheral blood showing hypochromia target cells oval cells and poikilocytes ×640

Table 1 and figure 3 show the results of findings in the other living members of the family including the parents of the patient her brothers and sisters and the third generation of her nieces and nephews in addition to two of three spouses of members of the second generation The patient s father (P O) had died at age 63 years two years before we saw the patient L O but he had been admitted to another hospital in 1942 where blood studies had been performed and where a diagnosis of duodenal ulcer and nonfunctioning gall bladder had been made His laboratory findings were made available to us and are recorded in table 1

A sister of the patient L O died at age 5 years of burns She was never investigated for the presence of anemia and was thought to have been entirely well All the other members of the family thought they were in good health



FAMILIAL MICROCYTIC ANEMIA

Fig 3 *Occurrence of thalassemia minor in three generations of a family of Italian origin Numbers after initials indicate age of patients in years*

TABLE I

Patient	Sex	Generation	Age	RBC × 10⁶	Hgb	WBC × 10	PCV	MCV	MCH	MCHC	Bizarre cells	Target cells	Retic	Spleen below costal margin	Icteric Index	Fragility Test saline Beg	Fragility Test saline Comp
					Gm			m	micro micro gms					cm			
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				3 90	10 4	7 110											
M O	F	1	66	5 50	12 0	5 900	40	72 7	21 9	30 0	+	+		0	8	0 46	0 30
L O	F	2	37	5 57	10 4	5 300	36	64 9	18 7	28 9	+++	+++		0		0 38	0 20†
R S	F	2	43	4 84	10 4	5 150	34 5	71 5	21 6	30 2	++	+		0 5	8	0 34	0
J O	M	2	45	6 33	12	8 500	45	71 0	20 1	28 2	++	++		2	2	0 42	0 20†
T S	F	2	31	6 53	1 9	10 900	4	62 7	8 2	29 1	+	+		1	8	0 38	0 20†
M S	F	2	34	5 33	11 0	10 200	38	1 4	20 8	29 2	+++	+++	0 1	0	5	0 36	0 20†
M S	F	3	21	6 99	12 2	6 700	38	54 4	17 4	32 1	+++	+	0	0 5		0 42	0 22†
R S	F	3	24	4 90	13 4	7 950	42 5	86 7	27 2	31 5	0	0		0 5		0 44	0 30†
R S	M	3	7	5 03	13 1	10 200	41	81 5	26 0	31 2	0	0	0 3	0	6	0 44	0 32‡
S S	M	3	10	6 42	11 5	7 300	40	6 3	17 8	28 7	+	+++	0 2	0	4	0 44	0 22†
W O	M	3	18	5 37	15 9	7 500	47 5	88 6	29 7	33 5	0	0		0		0 46	0 24‡
R O	F	S 2	38	4 68	13 4	6 500	44	94 0	28 7	30 4	0	0		0			‡
S S	M	S 2	39	5 36	15 6	6 500	46	86 8	29 1	31 7	0	0		0		0 44	0 32‡

* S 2 = spouse of member of second generation

† Platelets estimated from smear increased in number

‡ Platelets estimated from smear normal in number

She was seen again one month later with the same complaints and laboratory findings There had been no response to iron therapy Saline fragility test showed beginning hemolysis at 0 38 per cent and

molysis began at about normal concentrations 0 42 to 0 46 per cent saline indicating that a part of the population of erythrocytes had normal osmotic properties and were presumably of normal shape In some hemolysis began at much lower levels indicating that all of the erythrocytes were flatter than normal Complete hemolysis occurred at lower than normal concentrations in all cases ranging from 0 20 to 0 24 per cent with the exception of the mother who had complete hemolysis at 0 30 per cent only slightly below normal

In this series there was no increase in reticulocytes stippled erythrocytes or nucleated red cells The leukocyte and differential blood counts were normal in all of the affected individuals In some cases the number of platelets appeared to be slightly increased as estimated from the blood films Platelet counts were not made X rays of bones showed no specific lesions in the one case (L O) on whom they were made The anemia is completely resistant to iron therapy

Discussion

This asymptomatic, microcytic hypochromic anemia is not in itself of great importance except as the condition may fail to be diagnosed or diagnosed incorrectly Of significance however is its relation to thalassemia major a more severe disease usually fatal during childhood In this series two Italians with the mild form of the disease thalassemia minor produced 6 children 5 of whom are known to have had an identical mild form of the condition It would appear at first hand therefore that the trait dominant in both the parents was simply inherited by the children That such is not the case however has been demonstrated by the work of others and is further confirmed by the occurrence of the condition in the third generation of this series in which only 2 of 5 were affected

From the literature and from this series it seems probable that the severe form of the anemia thalassemia major results from homozygosity of an inherited factor while the milder form thalassemia minor results from heterozygosity of the same factor According to this idea both parents were heterozygous since they had the mild form of the disease They would be expected to have children who were homozygous (thalassemia major) heterozygous (thalassemia minor) and completely free of the trait in a ratio of 1 2 1 That all 5 of the second generation studied had thalassemia minor signifying heterozygosity was therefore apparently due to chance occurrence in a small sample The statistical probability of this occurrence being due to chance is 1 in 14 (P value about 0 07 borderline significance)

The third generation in this series however fits the idea very well In this case if one parent were heterozygous and the other free of the trait half the offspring would be expected to be heterozygous (thalassemia minor) while half would be expected to be unaffected The occurrence of thalassemia minor in 2 of 5 members of the third generation agrees with this concept

The mechanism of production of the abnormal erythrocytes is not understood Valentine and Neel[1] were able to produce target cells experimentally both in vitro and in vivo by increasing the tonicity of the solution in which the cells were suspended Whether this represents the natural mechanism of production of target cells has not been established

Summary

1 Three generations of a family of Italian descent were studied Nine of 13 members were found to have thalassemia minor

It will be noted (table 1 figure 3) that the parents of the patient all 5 living members of the second generation and 2 of 5 members of the third generation apparently had identical blood findings The number of target cells and bizarre forms varied somewhat but the essential features were identical A photomicrograph of the blood of S S a 10 year old nephew of the patient (L O) is shown in figure 4 and a photomicrograph of the blood of M S aged 21 a niece of the patient is shown in figure 5

The 9 affected members of this family were individuals with apparent good health capable of doing a full day s work without unusual fatigue There were no signs or symptoms which would have led to a diagnosis of a blood dyscrasia except the presence of palpable spleens in 5 of the 9 affected individuals

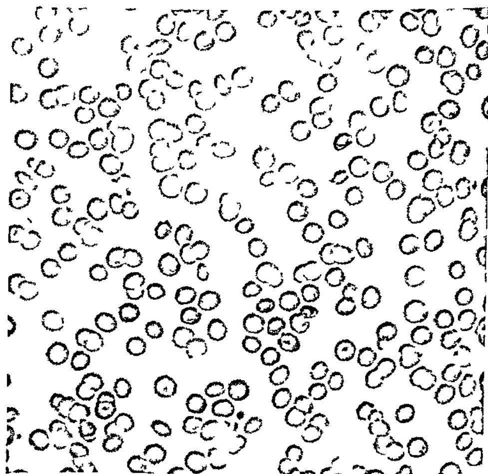


Fig 5 M S third generation Peripheral blood showing target cells oval cells and poikilocytes ×640

Thus such cases are usually disco ered coincidental to examination for other reasons or during a special study of a group of patients The individuals are of Italian or Sicilian descent The spleen is palpably enlarged in some cases but the enlargement is not great as compared with others of the blood dyscrasias and the finding is not constant Palpable enlargement of the li er did not occur in this series

The erythrocyte count is frequently elevated above normal and in this series was as high as 7 000 000 per cu mm The hemoglobin content and hematocrit values re low of course so that the mean cor puscular volume and mean corpusc lar hemoglobin alues are consider bly less than normal The mean corpuscular hemoglobin concentration is not reduced as much a further indication of the microcytosis Examination of the blood films confirm these data Hypochromia is obvious in addition to which ther are variable numbers of target cells and cells of bizarre shape Some of the smaller erythrocytes appear to be well filled with hemoglobin

The saline fragility test was abnormal in all 9 of the affected indi idual in this series In some he

A STUDY OF SICKLING OF YOUNG ERYTHROCYTES IN SICKLE CELL ANEMIA

By JANET WATSON M D

THE COMPARATIVE scarcity of sickled reticulocytes and normoblasts in patients with active sickle cell disease has been sufficiently striking to arouse comment by several students of that disease [1, 2, 3, 4] This observation led Murphy and Shapiro[3] to postulate that the occurrence of hemolytic crises might be due in part to the increasing tendency of the red cells to sickle on aging in which case the reticulocytosis of a crisis might of itself be beneficial The fact that their patient showed a higher percentage of sickle cells in the plain smear before crises than afterwards seemed to lend further support to this theory In reviewing many blood smears of sickle cell patients I was able to find only two normoblasts and one reticulocyte in the sickled form A quantitative study of the sickling of reticulocytes was therefore undertaken

MATERIAL

Three patients with active sickle cell anemia from the Hematology Clinic of Kings County Hospital were chosen for study because they consistently showed a high percentage of reticulocytes and sickle cells in their blood The Wintrobe oxalate mixture was used as an anticoagulant for the venous blood obtained

METHODS AND RESULTS

1 *Sealed Preparation*

(a) *Blood* Oxalated blood was mixed with equal parts of 0 5 per cent brilliant cresyl blue in 0 85 per cent NaCl solution One drop of the mixture was used to make the standard slide-cover slip preparation This was sealed with paraffin incubated at 37C and examined for sickling at frequent intervals In all cases sickling was complete within two to four hours This included the reticulocytes as well as the occasional normoblasts present It was the impression that most of the reticulocytes sickled as soon as the mature red cells with the exception of some of the most immature reticulocytes which were packed full of reticulum The normoblasts appeared to be the last to sickle The progressive sickling of the reticulocytes could not be counted accurately by this method however because of the irregularity of sickling in different parts of the same preparation This irregularity has been mentioned by others and is probably largely due to uneven distribution of the leukocytes which have an accelerating effect on the sickling of the red blood cells [5] presumably through lowering oxygen tension by metabolism Platelet distribution may also be a factor [6]

(b) *Bone Marrow* One cc of sternal marrow was obtained from one patient (W B) The buffy layer was used in a sealed preparation made as described above in order to study the sickling of normoblasts Cresyl blue stains the cytoplasm of the basophilic and polychromatophilic normoblasts but not that of the orthochromatic normoblasts unless it happens to be reticulated The reticular network is easily distinguished from the diffuse blue staining of the basophilic normoblasts It was found that none of the basophilic and polychromatophilic normoblasts were sickled after 24 hours incubation at 37C whereas most of the orthochromatic normoblasts were in the sickled state Two of the latter type containing Howell Jolly bodies however failed to sickle Since the orthochromatic normoblasts have a full quota of hemoglobin in their cytoplasm their ability to sickle is not surprising Further evidence for the primary role of hemoglobin in sickling has been presented recently by Ponder [7] who showed that when meniscocytes were made into ghosts by lysis of their hemoglobin they lost their ability to sickle

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2 Genetic studies indicate that this mild microcytic hypochromic anemia characterized by the presence of target and elliptical cells and other bizarre forms and by increased resistance to hypotonic saline results from heterozygosity of an inherited factor which when homozygous, produces thalassemia major or Cooley s Mediterranean anemia

3 If individuals heterozygous for this factor (thalassemia minor) marry other heterozygous individuals one quarter of the offspring can be expected to be homo zygous (thalassemia major) one half heterozygous (thalassemia minor) and one quarter free of the trait

4 The presence of thalassemia minor apparently did not interfere with the general health of affected members of this family and did not appear to shorten life expectancy The importance of the condition lies in its relation to thalassemia major

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cytes By comparing these figures with those for the percentage of total sickled cells it is evident that the rate of sickling of the reticulocytes is quite similar to the rate of sickling of the whole red cell population A reticulocyte in the sickled form is shown in figure 1

A possible theoretic objection to the fact that sickle cells are not reticulated in an ordinary smear is that the abnormal shape interferes with the supravital staining In answer to this objection blood was completely sickled in the test tube chamber after which an equal amount of saline cresyl blue previously aerated with carbon dioxide was introduced into the test tube under oil The blood was examined after two minutes and the sickled reticulocytes were found to be well stained

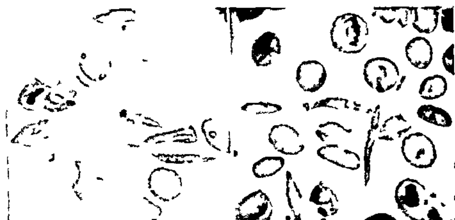


Fig 1 Fig 2

Figure 1 Meniscocytes sickled by carbon dioxide stained with cresyl blue and immediately counter stained with Wright s stain Cells are shown in the process of unsickling with transition forms from the sickled shape to the biconcave disk Note the sickled reticulocyte at the left Two crescent forms have developed elongated processes at the ends The black spots with refractile contours are artefacts in photography

Figure 2 Peripheral blood smear stained with cresyl blue and Wright s stain Three reticulocytes and seven crescent elliptical and oval shaped sickle cells can be seen None of the sickle cells are reticulated These sickled cells lack the bizarre shapes seen in Fig 1

In all three methods used sickling of all erythrocytes and normoblasts was quickly reversible within a few seconds upon admission of oxygen to the system

Discussion

Direct counting of the progressive sickling of reticulocytes on aeration with carbon dioxide (table 1) by means of a gas test tube chamber has shown that most reticulocytes sickle as well as the more mature cells However it was noted in this and in the other methods used that the reticulocytes with the largest amount of reticulum were usually late in sickling The few normoblasts observed were also somewhat slow Although this may be due to the possibility that the most immature cells have a lower oxygen tension threshold for sickling there is an al

2 *Gas Chamber Method*

The apparatus used was essentially that described by Hahn and Gillespie[8] except that the chamber was made of paraffin instead of glass A water sealed outlet was found necessary [4] Both carbon dioxide and nitrogen were used for sickling The same saline cresyl blue blood mixture was used Since the red cells in the hanging drop can be studied directly with the oil immersion lens this method has the advantage that the active dynamic process of sickling can be watched easily Here again most of the reticulocytes seemed to sickle as fast as the other red cells but the great rapidity of the sickling—about two minutes for complete sickling—made impossible the quantitative timing of the transformation of the two types of cells Rarely would the rearrangement of the hemoglobin in the process of sickling result in the reticulum being lost from view Occasionally the formation of the Sherman holly wreath forms of sickle cells[4] made this method as well as that of the sealed preparation unsatisfactory Because of these disadvantages a chamber method which would permit slower sickling and the periodic removal of cells for counting purposes was devised as follows

3 *Gas Test Tube Method*

A test tube 15 x 40 mm with a capacity of 5 cc was set up with a gas inflow and outflow via 24 gage needles through a rubber stopper The outflow was equipped with a water seal Carbon dioxide was used

TABLE 1 —*Progressive Sickling of Reticulocytes upon Aeration with Carbon Dioxide*

Patient	Time in CO_2	Reticulocytes (per 100 RBC)	Sickle Cells (per 100 RBC)	Sickled Reticulocytes (per 100 RBC)	Sickled Reticulocytes (per 100 reticulocytes)
	min				
L J	0	10.5	9.5	0.0	0.0
	2	10.0	45.0	4.5	45.0
	5	9.0	81.0	7.0	77.7
	10	9.0	92.5	8.5	94.4
W B	0	20.5	15.0	0.0	0.0
	2	19.5	28.0	4.0	20.5
	5	21.0	69.5	13.5	64.3
	10	20.0	90.0	17.5	87.5
J W	0	15.0	11.5	0.0	0.0
	2	16.5	33.5	4.5	27.3
	5	14.0	76.0	9.5	67.8
	10	14.5	93.5	13.0	89.6

for sickling One cc of the saline cresyl blue blood mixture was introduced into the inverted test tube Small samples of blood were removed at appropriate intervals under oil with an oiled tuberculin syringe and a 22 gage needle through the rubber stopper The blood was immediately injected into formalin for fixation Reticulocyte and sickle cell counts had to be done immediately in order to avoid inaccuracy due to slow fading of the reticulum in formalin A 2 per cent formalin solution in normal saline was found to be as good a fixative as the standard 10 per cent solution and had less of a fading effect A drop of the red cell suspension was placed on a slide under a cover slip and a count was made under oil immersion of the reticulocytes and sickle cells Only 200 cells were counted because of the time factor of fading

The data obtained are shown in table 1 The number of sickled reticulocytes found per 100 RBC was divided by the number of reticulocytes per 100 RBC in order to calculate the percentage of sickled reticulocytes in terms of total reticulo

not found in the irreversibly sickled shape As the maturing red cell stagnates in anoxic organs irreversible sickle forms appear and augment the vicious cycle of stagnation anoxemia increasing sickling thrombosis and hemolysis

Summary

1 Data have been presented to show that most reticulocytes from patients with the sickle cell trait or sickle cell anemia sickle as readily as do more mature red blood cells The most immature reticulocytes and normoblasts tend to sickle more slowly

2 Orthochromatic normoblasts were the only type of normoblasts which sickled the basophilic and polychromatophilic types could not be sickled

3 It is suggested that the sickle cell forms seen in ordinary stained smears represent old cells which have lost their elasticity while stagnating in the sickle shape and are unable to revert to a biconcave disk This would explain the fact that these forms are so rarely found to be reticulated when stained with brilliant cresyl blue

Acknowledgment

I wish to express my gratitude to Dr William B Castle Dr William Dock (Department of Medicine) for many suggestions to Dr John M Pearce (Department of Pathology) for assistance in preparing this report and to Mrs Muriel MacDowell (Department of Pathology) for the photomicrography

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ternative explanation that the presence of a nucleus or of a large quantity of stained reticulum may mechanically interfere with the sickling process

The virtual absence of reticulated sickle cells in the ordinary smear (fig 2) seems paradoxic at first glance The sickle cells seen in the fixed blood smear appear different from the ones produced by in vitro sickling [6, 9] The former are crescent shaped elliptic or oval and do not have the long processes seen in sickled preparations When viewed directly in a hanging drop these forms are seen to send out one or more elongated processes at the pointed ends on aeration with carbon dioxide or nitrogen and to revert to their original shape on aeration with oxygen without going on to the normal discoid forms For this reason these abortive sickle cells should perhaps have a distinctive terminology It may be that they are old cells that have lost their elasticity from being kept in the sickled shape for long periods of time in stagnant blood vessels Good evidence for the role of stagnation is presented by Diggs and Bibb who had three patients with sicklemia who had *irreversible sickle cells in their pleural or ascitic fluid although they had none in* their stained blood smears It should be possible to produce these crescent forms in vitro by keeping cells in their sickled shape for long periods of time but attempts so far have been unsuccessful * It is a well known fact that sickle cells are not seen in the blood smears of persons with the sickle cell trait This could be predicted since the oxygen tension necessary for in vitro sickling (18 mm Hg)[4] would never occur in vivo

Reticulocytes being young cells may have more elasticity so that they are not fixed in the sickled shape in vivo Or it may be that the length of time for stagnation of erythrocytes to produce these abnormal forms may surpass the estimated five to six day life span of the reticulocyte [10] If we accept Tomlinson s finding[11] that *sickled cells are actually stuck in the interstices of the spleen pulp and cannot* be perfused out it would seem that the spleen could be an important dragnet of sickled erythrocytes in those patients in whom that organ had not become entirely fibrotic

There is disagreement as to whether the number of circulating sickle cells differs from time to time in the same patient Diggs and Bibb and Smith[12] found little variation while Sydenstricker[13] and Murphy and Shapiro[3] found that the sickle cells increased before the crisis and fell after the onset Emmel[14] also noted significant variation in his patient The high viscosity of sickled cells[15] and their abnormal shape tend toward their sequestration in organs so that there could be an increasing accumulation of sickled forms without this increase necessarily being reflected in the peripheral blood If this is so the diverse reports among various *investigators is not surprising The increased mechanical fragility of the* sickled cells [16] must be an important factor in their final demolition Thus a crisis results in the destruction of the old sickled cells and in the outpouring of new young red cells Although the reticulocytes appear to sickle as well as other cells they are

* A personal communication from Dr Shu Chu Shen indicates that sterile incubation in vitro of erythrocytes maintained in the sickled form renders them unable to reassume the discoid form upon exposure to oxygen The technic employed was the sterile incubation of defibrinated blood samples from either anemic patients or those showing the trait for only twenty four hours This incubation was carried out after preliminary equilibration with and during continuous exposure to a gas mixture composed of 90 per cent nitrogen and 10 per cent carbon dioxid

When she was admitted to the Hospital on May 1 1941 the hemoglobin value was 11 05 Gm per 100 cc the erythrocyte count was 3 140 000 the leukocyte count was 7 500 and the reticulocytes numbered 17 6 per cent Examination of a blood smear showed the picture of congenital hemolytic icterus (micro spherocytosis) The serum bilirubin value (indirect reaction) was 0 9 mg per 100 cc of serum The brom sulfalein test for liver function did not show any retention of the dye The fragility of the erythrocytes was normal initial hemolysis occurred at a concentration of sodium chloride of 0 44 per cent with complete hemolysis at 0 32 per cent Splenectomy was performed May 5 1941 the spleen weighed 310 Gm The patient had a stormy convalescence but was dismissed June 21 1941 without having been given any transfusion At that time the hemoglobin value was 10 6 Gm and the erythrocyte count was 3 700 000

TABLE 1 —*Hemolyti Anemia with Significant Microspherocytosis*

Case	Age	S	Hemo-gl b (Gm per 100 cc)	Erythrocytes: ✱ p r c mm	Erythrocytes: F ag l ty	R t c ul cytes	Gall t nes	Time f l lowed	H m glob pe 100 cc	Erythrocytes ✱ pe mm	Result
			B f e spl t my					*Afte spl tomy*			
	y s				%	%		mo			
1	26	F	3 9	1 390 000	0 46 0 38	25	0	13		4 100 000	Improvement
2	30	F	6 0	1 530 000	0 50–0 38	62	0	30		4 000 000	Excellent
3	35	F	11 0	3 140 000	0 44 0 32	18	+	12	8 3 Gm	2 740 000	Poor†
4	39	F	8 4	2 520 000	0 50–0 38	51	+	1	10 8 Gm	4 520 000	Improvement
5	39	F	8 3	2 710 000	0 46–0 32	32	0	9	84%	3 537 000	Excellent
6	54	F	8 4	2 900 000	0 46 0 36	24	+	26	88%		Excellent
7	59	F	6 3	3 850 000	0 48 0 36	3	0	12	12 5 Gm	3 850 000	Excellent
8	60	M	6 8	2 000 000	0 50 0 34	37	0	24	90%	5 000 000	Excellent
9	61	F	4 9	1 640 000	0 66–0 40	40	0	4	13 6 Gm	3 760 000	Excellent†
10	65	F	6 0	1 520 000	0 50–0 36		0				Died 8th post operative day Trans fusion reaction
11	74	M	4 3	1 150 000	0 50–0 36	27	0	24	60%	2 800 000	Fair

In hypotonic solution of sodium chloride
† Reported in detail in text

When the patient returned to the clinic on August 11 1941 the hemoglobin value was 10 6 Gm the erythrocyte count was 3 430 000 and the reticulocytes numbered 6 5 per cent On September 29 1941 the hemoglobin value was 10 Gm the erythrocyte count was 2 610 000 and the reticulocytes numbered 24 2 per cent The fragility of the erythrocytes had increased initial hemolysis occurred at 0 5 per cent and was complete at 0 36 per cent On May 9 1942 the hemoglobin value was 8 3 Gm the erythrocyte count was 2 740 000 and the reticulocytes numbered 28 8 per cent The value for the indirect serum bilirubin was 1 8 mg Examination of blood smears revealed typical microspherocytosis and increased regeneration of erythrocytes

In a letter dated February 7 1945 the patient stated that her hematologic picture was about the same as it had been before splenectomy was performed

Case 3 illustrates the failure of splenectomy to relieve the anemia Of interest in this case are the presence of normal fragility prior to splenectomy and an increase in fragility after removal of the spleen Despite the absence of a family history of hemolytic anemia in this case the blood picture was considered typically that of hemolytic icterus by several observers

PRIMARY NONFAMILIAL HEMOLYTIC ANEMIA

By J M Stickney M D, and Frank J Heck, M D

ALTHOUGH patients with hemolytic anemia are not numerous they continue to be a problem of special interest and great difficulty In the majority of cases the disease is of the familial or congenital type The commonly accepted criteria for the diagnosis of congenital hemolytic anemia include the presence of a microspherocytic blood picture with an increase in signs of regenerative activity increased fragility of the erythrocytes in varying concentrations of hypotonic saline solution splenomegaly an elevated value for indirect serum bilirubin with an increased excretion of fecal urobilinogen and a history of anemia icterus splenomegaly or increased fragility of erythrocytes in other members of the patient s family

In the differential diagnosis of the different types of hemolytic anemia the question not infrequently arises as to whether an individual instance of the disease should be regarded as belonging to the congenital or familial type or to the acquired type As Watson pointed out there has been a tendency to regard all instances of primary hemolytic jaundice as of familial or congenital type There are, however no clear cut criteria to which all writers on the subject agree In some cases in which the family history is negative but other criteria are present the disease is classified as acquired It must be admitted that a negative family history is not a definite indication that the disease is of the acquired type since actual investigation of close relatives may reveal such changes as increased fragility of the erythrocytes in the absence of other findings

In the years 1942 through 1946 at the Mayo Clinic splenectomy was performed in 22 cases of hemolytic anemia in which no positive family history could be obtained These 22 cases are the object of our special interest * As far as could be determined the hemolytic syndrome in these cases was not secondary to any toxic infectious or poisonous agent and was not symptomatic and part of a primary disease such as lymphoblastoma leukemia or hepatic cirrhosis

We have divided these cases into two groups which happen to be equal in number In the first group either microspherocytosis or increased fragility of the erythrocytes or both were found In the second group such evidence was not present The groups are summarized in tables 1 and 2

Report of Selected Cases

Case 3 The patient was a married woman aged 35 There was no family history of anemia jaundice or splenomegaly In November 1940 she complained of weakness and malaise She was yellowish and was found to be anemic On March 10 1941 the erythrocytes numbered 790 000 and the leukocytes numbered 77 750 per cubic millimeter of blood Many transfusions were given over a period of one month with moderate benefit Physical examination disclosed that the spleen was enlarged and extended about 1½ inches below the costal margin

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* In the years 1942 through 1946 a diagnosis of congenital hemolytic icterus was made in 115 cases at the Mayo Clinic Splenectomy was performed in approximately 90 of these cases

weighed 1 5-0 Gm The postoperative convalescence was uneventful and no transfusion of blood was administered The amounts of urobilinogen excreted in the feces were as follows on December 17 and 18 230 mg per twenty four hours on December 19 and 20 85 mg per twenty four hours on December 21 and 22 45 5 mg per twenty four hours

On December 23 the value for the indirect s rum bilirubin v as 0 6 mg When the patient was dismissed on January 4 1947 th h moglobin valu v as 8 7 Gm and th erythrocyt s numbered 2 900 000

In a letter dated March 17 194- the patient stated that her blood picture had improved The hemoglobin value was 13 6 Gm the erythrocyte count was 3 -60 000 and the leukocyte count was 11 600

In case 9 which belongs to the microspherocytic group the result has been excellent In this group the results of splenectomy as a whole were considered good In 9 of the 11 cases the patients were women There was one death in this group and in one case (case 3) the patient was not improved In case 11 the patient was improved but did not obtain an excellent result

Case 13 A boy aged 11 came to the clinic July 31 1942 He had had intermittent attacks of jaundice since the ag of 2 when he had had a f brile illness which had been diagnosed as Malta f ver The ordinary contagious diseases of childhood such as whooping cough measles and scarlet fever each had been followed by jaundice The jaundice also had occurred after an infection of the upper part of the respiratory tract

When the patient was examined at the clini he was slightly icteric and the spleen could be palpated 2 inches below the costal margin The hemoglobin value was 10 5 Gm the erythrocyte count was 3 270 000 with 10 per cent reticulocytes and the l ukocyte count was 5 400 Examination of a blood smear disclosed acti e regeneration of the erythrocytes but no microspherocytosis The indirect serum bilirubin value was 2 2 mg and the bromsulfalein test for liver function disclosed no dye retention The fragility of the erythrocytes was normal It was felt that the patient had hemolytic anemia o an acquired type Splenectomy was ad ised

The patient returned to the clinic May 25 1943 In August 1943 he had had an attack of epigastric pain He had been markedly jaundiced and his temperature had reached 103 F After ten days he had made a rapid recovery Examination disclosed hematologic findings as they were at the time of the patient s first visit to the clinic with the exception that the indirect serum bilirubin value was 5 5 mg Splenectomy was performed on May 29 The spleen v eighed 266 Gm When the patient was dismissed the value for the hemoglobin and the erythrocyte and leukocyte counts were the same as they had been when the patient came to the cl nic but the indirect serum bilirubin value had dropped to 0 5 mg

He returned to the clinic July 19 1944 because of an attack of pain in the upper part of the abdomen and jaundice At this time the hemoglobin value was 12 3 Gm the erythrocyte count was 3 880 000 and the leukocyte count was 11 800 Macrocytosis of the erythrocytes was observed for the first time No change in the hematologic findings was observed when the patient was seen again on August 8 1946 Attacks of jaundice had continued to occur The patient s growth has continued in an apparently normal manner

Case 15 A married woman aged 21 came to the clinic April 20 1945 In January 1944 she had had a miscarri ge at the fourth month of pregnancy During the pregnancy her parents had thought that she had appeared yellow After the miscarriage she had lost weight and h d become very weak She had been treated for anemia There was no family history of hemolytic anemia Serologic tests for syph lis on both the blood and spinal fl id had been strongly positi e and antisyphilitic treatment had been admin istered intram scularly There was no person l or family history of syphilis and the patient denied the possibility of contact infection Between March 27 and April 12 1945 she was given a total of 2 500 cc of blood in nine transfusions She h d been told that her hemoglobin value was lo er after these trans f sions than it had been previously

When she v as examined at the clinic the hemoglobin alue was 8 8 Gm the erythrocyte count vas 2 520 000 and the leukocyte count was 8 200 Examination of a blood smear reve led a m rked increase in the regeneration of th erythrocytes and a regenerati e macrocytosis There was abundant myeloid

Case 9 A married woman aged 61 came to the clinic on December 3, 1946. She had been perfectly well until the previous summer, when she had noted a loss of strength and loss of appetite. In the fall of 1946 she had become very thirsty. On October 31 she had been admitted to a hospital because of diabetic coma. The blood sugar value was 310 mg. per 100 cc. The presence of diabetes had not been recognized previously. Hematologic examination had disclosed severe anemia; the hemoglobin value had been found to be 5.6 Gm. She had not been jaundiced previously and there was no family history of anemia, jaundice or splenomegaly. The diabetes had been controlled by dietary measures and by the administration of insulin. She had received eight *transfusions of blood* (*500 cc. at each transfusion*). *The last transfusion* had

Table 2.—*Hemolytic Anemia without Significant Microspherocytosis*

Case	Age	Sex	Before splenectomy: Hemoglobin, Gm. per 100 cc.	Before splenectomy: Erythrocytes, No. per cu. mm.	Before splenectomy: Erythrocytes, Fragility*	Before splenectomy: Reticulocytes	Before splenectomy: Gallstone	After splenectomy: Time followed	After splenectomy: Hemoglobin per 100 cc.	After splenectomy: Erythrocytes per cu. mm.	Result
	yrs.				%	%		mos.			
12	4	F	8.1	2,770,000	0.40-0.28	13	0	24	5.8 Gm.	2,380,000	Poor. Explored for accessory spleen
13	13	M	10.3	3,270,000	0.44-0.30	10	+	24	10.6 Gm.	2,980,000	Poor†
14	19	F	4.3	800,000	0.46-0.36	40	0	12		3,750,000	Excellent
15	21	F	8.8	2,520,000	0.44-0.34	32	0	23	78%	4,120,000	Excellent†
16	24	F	11.9	4,100,000	0.44-0.32	5	+	4	13.8 Gm.		Improvement
17	33	M	6.3	2,220,000	0.44-0.32	17		40	14.8 Gm.		Excellent‡
18	34	F	11.6	3,350,000	0.42-0.30	12	+	18	78%	3,400,000	Fair. One episode of jaundice
19	40	F	4.9	1,560,000	0.44-0.30	30	0	4	11.0 Gm.		Died of intestinal obstruction
20	46	F	8.1	3,850,000	0.48-0.28	3	+	1	11.05 Gm.	4,920,000	Unknown
21	54	F	5.5	1,340,000	0.44-0.30	30	+	12	12.9 Gm.	4,050,000	Excellent
22	59	F	4.7	1,520,000	0.44-0.32	31	0	4	10.2 Gm.	4,000,000	Died of serum hepatitis

* In hypotonic solution of sodium chloride.
† Reported in detail in text.
‡ Course following splenectomy reported through courtesy of Dr. C. J. Watson.

been administered on November 29. Despite the lack of clinical evidence of transfusion reactions, the anemia had not improved.

When the patient came to the clinic on December 3, the hemoglobin value was 4.9 Gm., the erythrocyte count was 1,640,000 and the leukocyte count was 11,200. Examination disclosed moderate icterus. The spleen was greatly enlarged and extended downward to the crest of the ilium. Examination of blood smears disclosed marked microspherocytosis, very active regeneration of erythrocytes and 40 per cent *reticulocytes*. The serum bilirubin values were 1.4 mg. direct and 3.9 mg. indirect. A fragility test showed initial hemolysis at 0.66 per cent and complete hemolysis at 0.36 per cent. The bromsulfalein test for liver function did not disclose any dye retention. During the first forty-eight hours in the hospital the average *amount of fecal* urobilinogen excreted each twenty-four hours was 4,800 mg.; during the next forty-eight hours this averaged 1,220 mg. No irregular agglutinins were demonstrable.

The diabetes was carefully controlled. Splenectomy was performed on December 13. The spleen

spherocytic it is possible that this antibody titer may have been the cause of the hemolytic anemia In any event improvement did not occur until splenectomy was done At the present time a more intensive search for irregular agglutinins and hemolysins is being carried out in certain cases of hemolytic anemia [5]

Several authors have emphasized the dangers of severe hemolytic reactions following blood transfusion in hemolytic anemia In one of our cases (case 10) death was probably due to a hemolytic transfusion reaction after operation We have not noted any severe exacerbation of the hemolytic process in the other cases but we have been impressed with the failure of transfusion to benefit the patient especially before splenectomy

We have found it difficult to correlate the degree of anemia with the severity of the jaundice In one case (case 4) as long as the patient was severely jaundiced the anemia was relatively mild When the severity of the jaundice decreased the concentration of hemoglobin decreased rapidly This inverse relationship has been noted by Watson and Fowler

In several of our cases bone marrow was examined A definite hyperplasia of the normoblastic cells was seen in each instance No megaloblasts were found

Although not common leukopenia and thrombocytopenia may accompany the anemia Doan and Wright have recently reported this phenomenon as a panhematopenia In case 14 the number of leukocytes ranged from 3 100 to 5 000 and the number of thrombocytes from 65 000 to 75 000 per cubic millimeter before splenectomy Both were normal or increased in number after operation

In a case not included in this series splenectomy was performed for what appeared to be a primary hemolytic anemia The blood picture was subsequently that of chronic myelogenous leukemia At the time of the original examination there was not as much myeloid immaturity as there was in the blood of many of the patients in the present series The sternal marrow was hyperplastic and could not be distinguished from nonleukemic hyperplastic marrow

Splenectomy may be of definite benefit in symptomatic hemolytic anemia when the progres of the primary disease is not rapid Recently a woman who was 66 years of age came to the clinic because of weakness of six months duration The hemoglobin value was 8 3 Gm the erythrocyte count was 2 250 000 with 15 per cent reticulocytes A spleen which weighed 1 125 Gm was removed and a diagnosis of follicular lymphoblastoma was made There were no enlarged lymph nodes One year later the patient who had regained her good health returned because of enlarged axillary and inguinal lymph nodes The hemoglobin value then was 11 7 Gm and the erythrocyte count was 4 150 000 Biopsy of a lymph node confirmed the previous diagnosis and for the first time roentgen therapy was started The splenectomy had relieved the weakness and anemia

Summary

In our experience in half of the cases of primary hemolytic anemia in which there is no family history of anemia jaundice or splenomegaly examination of the blood disclosed microspherocytic erythrocytes and increased fragility of erythrocytes The results of splenectomy in these cases are better than in those in which

immaturity but no evidence of microspherocytosis The indirect serum bilirubin value was 1 6 mg The fragility test revealed that hemolysis began at 0 44 per cent and was complete at 0 34 per cent The bromsulfalein test disclosed no dye retention The Kline Kahn Hinton and Kolmer serologic tests for syphilis were negative

On May 2 the hemoglobin value was 4 5 Gm the erythrocyte count was 1 4 0 000 with 32 2 per cent reticulocytes and the leukocyte count was 7 500 The amounts of urobilinogen excreted in the feces were as follows On May 1 and 2 894 mg per twenty four hours on May 3 and 4 642 mg per twenty four hours A transfusion of 500 cc of blood was administered on three occasions between May 3 and May 9 Splenectomy was performed on May 9 The spleen weighed 670 Gm Another transfusion of blood was given on May 21

The patient was greatly improved when she returned to the clinic for examination on October 24 1945 The hemoglobin value was 12 7 Gm the erythrocyte count was 4 620 000 with 8 6 per cent reticulocytes and the leukocyte count was 12 900 In the blood smear there was active regeneration of the erythrocytes with many macrocytes The amount of urobilinogen excreted in the feces was determined for a period of four days The average amount was 147 mg per twenty four hours The indirect serum bilirubin value was 0 45 mg

On March 25 1947 the patient s family physician informed us that the hemoglobin value was 78 per cent and that the erythrocyte count was 4 120 000

In the group of cases without significant microspherocytosis females again predominated The higher incidence of this disease among females has also been noted by Fowler In this group the results have not been as good as they were in the microspherocytic group although the number of cases is not large enough to draw definite conclusions However the results are encouraging enough to warrant further trial of splenectomy A longer period of observation is desirable to determine how frequently hemolytic episodes may occur after operation

Comment

In recent years several excellent reviews dealing with hemolytic anemia have appeared [1 2 7] Watson has classified the hemolytic anemias as microcytic (familial or congenital) and macrocytic (secondary or acquired) He stated that in all cases of the acquired type of the disease the erythrocytes are at least slightly larger and often much larger than the normal Dameshek and Schwartz and Singer and Dameshek pointed out that in some cases of acquired hemolytic anemia spherocytosis and increased hypotonic fragility are present although a pseudomacrocytic blood picture may be seen Fowler found that spherocytosis was not consistently present in a group of cases of acquired hemolytic anemia and that macrocytosis was more frequently encountered

All of our cases were examples of primary nonfamilial hemolytic anemia so far as we could determine Microspherocytosis was not present in half of these cases but with one exception (case 13) we could not classify them as cases of macrocytic anemia There was a considerable number of macrocytes in some of the smears but many of them were regenerative or polychromatophilic erythrocytes

Agglutinins and hemolysins may be etiologic factors in a hemolytic syndrome In two of our cases (cases 14 and 22) iso-agglutinins of an abnormal type were present In each instance the patient s serum agglutinated his own erythrocytes In another case (case 4) an Rh negative patient had a high Rh antibody titer due to previous transfusions of Rh positive blood Although the blood picture was micro-

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AUTOHEMAGGLUTININS AND HEMOLYSINS WITH HEMOGLOBINURIA AND ACUTE HEMOLYTIC ANEMIA IN AN ILLNESS RESEMBLING INFECTIOUS MONONUCLEOSIS*

By LAURENCE B ELLIS, M D OSCAR J WOLLENMAN M D AND RICHARD P STETSON M D

ACUTE acquired hemolytic anemia is uncommon but dramatic in its clinical picture and often disastrous in its outcome In 1940 Dameshek and Schwartz[1] assembled about 100 cases reported in the literature since 1907 in which no definite etiology was evident In addition as cited by these authors cases have been reported in which the anemia developed in association with definite or probable etiologic factors These include malaria, infections with streptococci staphylococci and certain anaerobic organisms as well as tuberculosis syphilis and anchylostomiasis also pregnancy lymphoma, leukemia carcinomatosis and finally various drugs especially the sulfonamides but also arsenical preparations phenylhydrazine and acetanilide Hemolytic anemia has also been reported in association with atypical pneumonia of unknown etiology [3] and its occurrence in patients who have received sulfonamides is well established [4] Studies of these cases in regard to the presence of hemagglutinins and hemolysins have given varied results

The case of acute hemolytic anemia with hemoglobinuria which is herewith reported is of interest because of the association of the unusual combination of autohemagglutinins and hemolysins occurring in the presence of morphologic changes in the white blood cells and heterophile agglutinins in the blood serum consistent with infectious mononucleosis together with a positive Donath Landsteiner reaction and the absence of evidence of syphilis

The patient was observed during the acute stage of his illness by two of the authors (O J W and L B E) and more than two years later by the third author (R P S)

REPORT OF CASE

Pres t Illness H J H a 21 year old single white male was admitted to an Army hospital in the European Theater on January 9 1945 with a three day history of bloody urine Two weeks prior to admission he had developed an upper respiratory tract infection with a nonproductive hacking cough headache moderate nausea and anorexia There was no vomiting until the day before admission when he had vomited once without hematamesis On the evening of January 6 he first noticed dark bloody urine unaccompanied by urgency frequency dysuria or nocturia The dark urine continued for the three days prior to admission During this interval he developed a steady aching pain in the epigastrium and in the lumbar region General weakness and dyspnea on exertion became moderately distressing but he continued on duty until admission At no time did he experience chills fever icterus or symptoms referable to the lower intestinal tract His weight had decreased from his usual 128 pounds to 105 pounds on admission

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From the Thorndike Memorial Laboratory and the Second and Fourth Medical Services (Harvard) of the Boston City Hospital and the Department of Medicine Harvard Medical School

This patient was observed from January to March 1945 in an Army Hospital in the European Theater of Operations and subsequently in 1947 in a Veterans Administration Hospital

microspherocytosis is absent True macrocytosis was observed in only one instance Females predominated in both groups of cases Agglutinins and hemolysins have not appeared to play any significant role in the production of the hemolytic syndrome in our cases We do not feel justified in expressing an opinion as to whether the microspherocytosis indicates a familial or congenital blood disorder From a practical standpoint it makes no great difference since splenectomy should be considered seriously in any case of chronic primary hemolytic anemia It may be of value in some cases of secondary or symptomatic hemolytic anemia

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This patient was observed from January to March 1945 in an Army Hospital in the European Theater of Operations and subsequently in 1947 in a Veterans Administration Hospital

Past History In 1937 he had an uncomplicated appendectomy in 1942 a hemorrhoidectomy On July 21 1944 he was admitted to an Army General Hospital with lymphadenitis of the right arm and was discharged well on Augu t 10 On August 24 1944 he developed impetigo and was treated with *ammoniated mercury and ultra violet light until* September 16 1944 when he was discharged as well From S ptember until the present illness approximately four months later he enjoyed good health In November 1944 his attention had first been called to asymptomatic enlargement of his finger tips which had not changed since to his knowledge

During the few days immediately preceding entry he had developed a generalized pruritic skin lesion which on admission proved to be scabies During his three years of army service he had never had tropical service and he had not been exposed to any known hemolytic agents H had received no sul fonamide drugs for at least six months prior to his illnes His diet had been adequate Alcohol had not been used habitually nor in excess immediately prior to the present illness There was no history of vene real disea e He had never experienced an illness similar to the present malady nor had he ever been seriously ill

Family History No similar illness was known The familial history was noncontributory

Physical Examination Temperature 99 4 F pulse 110 respiration 20 blood pressure 120 mm of mer cury systolic and 70 mm diastolic He was ambulatory and complained chiefly of weakness He did not appear acutely ill but was pale and slightly icteric Scabetic furrows and scratches were pres nt over the trunk and extremities (Subsequent treatment for scabies led to rapid clearing) The mucous membranes were moderately pale but presented no evidence of hemorrhage or ul eration There was a nontender left infra auricular lymph node approximately 1 cm in diameter No other lymphadenopathy was de tected The thyroid was not enlarged The chest was symmetrical and the examination of the lung was normal The heart was not enlarged There was a strong apical impulse and a blowing apical systolic murmur was heard on auscultation The abdomen was scaphoid with an indefinite epigastric tenderness The liver and spleen were palpable only on inspiration and were tender The kidneys could be palpated but were not enlarged or tender The genitalia were normal The fingers presented a striking terminal enlargement characteristic of clubbing the nails appeared otherwi e normal The toes did not show similar changes The extremities were not cold or sweaty Examination of the long bones skull the muscular and the neurologic systems was not remarkable There was no demonstrable edema or evidence of dehydration

LABORATORY DATA

Methods Employed Referen es are given below to the technical method employed in the laboratory examinations

Urine Examination hemo iderin (Rouss technic[6]) porphyrins[6] alkapton bodies[6] bile (*Rosenbach s* modification of Gmelin s test[6]) urobilinogen (Wallace and Diamond modification of Ehrlich aldehyde test[6]) hemoglobin in plasma and urine indican (Obermayer s test[6])

Blood Examinati n hemoglobin (alkaline hematin method using a Coleman spectrophotometer) red cell fragility[6] erythrocyte count (*Hayem* s solution) platelet count[6] bleeding time (*Duke s method*[6]) clotting time[5] hematocrit and sedimentation rate (Wintrobe method[6]) test for sickle cell trait [6]

Se ologic Examination het rophile agglutination presumptive test (Davidsohn technic[10 11]) Don ath Landsteiner[9] hemolysis test with acidified serum[7] cold hemagglutinins [12]

Other methods employed were either too well known to require comment or are described in the t xt

Hematologic data during this hospitalization are given in table 1 and figure 1

RESULTS

1 *Anemia* The anemia was obviously hemolytic in type as evidenced by extreme hemoglobinuria and became profound on the day following admission the hemo globin dropping from 12 2 grams per cent at 10 00 A M to 5 9 grams per cent by 3 00 P M of the same day At this time blood platelets were 232 000 bleeding time one minute clotting time five and one half minutes (capillary tube) and clot re

TABLE 1

Date	HGB	RBC	WBC	Differential count						Reticulocytes	Hematocrit	Serum Bilirubin	Heterophile Agglutination	Cold Agglutination (1 O Cell)	Urine				Remarks
				St b	Seg	Eos	Bas	Lym	Mon						Hgb	Alb	Bile	Urobilinogen	
1945	Gm %	mil	× 1000	%	%	%	%	%	%	%	%	Gm %							
Jan																			
9															4+	3+	1+		
10 (AM)	12 2	3 30	24 3		32	2		63	2			2 4			0	2+	1+		NPN 28 mg % Bl Sugar 112 mg % lymphocytes atypical
10 (PM)	5 9	clumped	15 7								22		1 1024	1 256	0	1+	1+		Acid hemolysis and sickling neg Donath Landsteiner positive
11	7 1	2 24	21 0	10	38			52		1 3	25	3 8		1 128	0	1+	1+		Blood Kahn and Wassermann neg
12	8 6	2 90	8 3	2	48			49	1	1 1	26				4+	3+	1+		*Fragility of RBC normal*
13	10 6		21 3							0 8					0	1+		1 40	
14	9 4	2 74	15 6	4	36	2		56	2	3 0	28	3 2	1 1024	1 64	0	1+	0		
16	9 8	2 83	13 6	3	34	3		51	9	5 0	29	3 2			0	0	0	1 40	Sed of RBC 1 mm/min
18	9 0	2 81	9 5	2	38	4	3	48	5		28			1 64	0	0	0		
19	7 7	2 21	15 6	3	44			50	3				1 1024		0	0	0		
21	5 6	1 63	9 7	2	34			64		4 4					0	0	0	1 160	
24	9 1	2 69	10 8	4	55	4		34	3	4 0		1 5			0	0	0	1 160	Total prot 9 1 Gm % alb 5 8 glob 3 3
Feb																			
1	9 4	3 16	8 5	1	65	2		18	3	5 2					0	0	0	1 20	
3	10 0	2 83	6 6		58	3	1	36	2		30	1 2	1 256	1 64	0	0	0	0	
10	11 2	3 40	[illegible] 0	1	1	1	1	4	2	9 8	35	1 0	1 128		0	0	0		Fragility of RBC normal
13	11 0	3 52	8 5	4	65	1		29	1	4 6	38		1 128	1 64	0	0	0		BMR +22
17	13 0	3 9	5 2	2	50	1	3	44		2 1	37				0	0	0		Donath Landsteiner pos MCV 92 MCH 31 MCHC 28
Mar																			
7	11 4	3 75	9 2		69	1	1	26	3	2 6					0	0	0		
	13 3	4 05								2 2	37				0	0	0		See Tests—Tables 4 and 5
1947 Feb	16 0	4 30	11 0		63	1		26	10	0 9		0 3	1 4	neg		0	0		Donath Landsteiner neg

1 64 [illegible] cells

traction normal The osmotic fragility test of the patient s red blood cells was identical with the control on the second hospital day on three subsequent oc casions within the next month and two years later in 1947 During the first two days difficulty was encountered in performing erythrocyte counts due to clumping of the cells in the pipet at the room temperature of approximately 20 degrees Centi

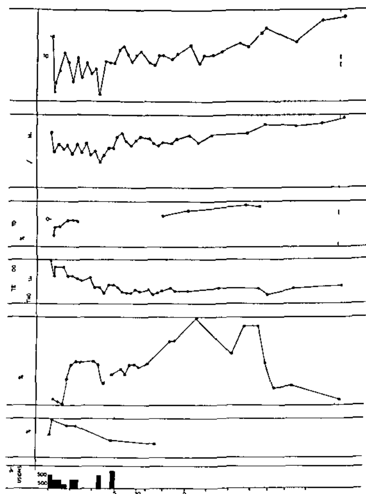


FIG 1

grade Subsequently warm saline solution was employed as a diluent and counts were then satisfactorily made

The course of the anemia is shown in figure 1 Therapy consisted of transfusions of whole blood and concentrated red cells as described below iron therapy and a high caloric high vitamin diet

2 *Morphology of the Blood* Examination of the morphology of the erythrocytes was not striking The anemia was essentially normocytic and normochromic and the red blood cells appeared morphologically normal No nucleated red cells were

observed The reticulocytes rose during the first and second week as shown in figure 1 There was an initial leukocytosis on January 10 of 24 300 with a differential of segmented polynuclear cells 32 per cent eosinophiles 2 per cent lymphocytes 64 per cent monocytes _ per cent The lymphocytes were typical of the cells seen in infectious mononucleosis the majority conformed to Downey s Class I[13] a small percentage were Class II and III The leukocyte count did not exceed 10 000 per cubic millimeter after the second week and the relative lymphocytosis with the atypical lymphocytes gradually disappeared

3 *Hemagglutinins and Hemolysins* On admission hemagglutinins in the patient s serum with red cells of blood group O were demonstrated in a dilution of 1 256 at 4 6 degrees Centigrade Clumping also occurred at 10 and 20 C and persisted when the test was carried out in the incubator at 37 C When preparations which previously had been chilled at 4-6 C were subjected to a temperature of 37 5 C for one hour the clumping persisted and hemolysis occurred There was no hemolysis at 4-6 C After subjecting the patient s serum to a temperature of 56 C for inactivation of serum complement hemolysis was not observed but agglutination reactions were

TABLE 2 —*P es mpti e Donath Land te ner Test*

T mp t Co ditio s	*Pl ma Hemoglob of D fib ted Bl d*	
	Pat e t	Co trol
	mg p 100 c	*mg p 100 c*
Room temperature	70	21
1 hr at 37 5 C	90	21
20 min at 4 6 C then 1 hr at 37 5 C	3 0	21

* Two cc samples of defibrinated blood from the patient and a normal control were treated as indicated

maintained The hemagglutination against red cells of blood group O dropped to 1 64 by January 14 where it remained throughout this hospital admission

The presumptive (table 2) and more complete Donath Landsteiner tests (table 3) were strongly positive on admission guinea pig serum was not available for addition as a source of complement to the heat inactivated serum Donath Landsteiner tests were repeated seven weeks later with the same positive result The Kahn and Wassermann tests were negative on four occasions during this month and again in December 1945 and January 1947 and there was no history or physical sign of syphilis

The relationship between chilling of the patient and the development of increased hemoglobinemia and hemoglobinuria was explored but unfortunately without conclusive results During his hospitalization the patient was exposed to the usual environment of a drafty Nissen hut ward heated by coke stoves in England in the winter During the first day of his stay he was a bed patient but was rather recalcitrant and difficult to keep in bed The opportunity for chilling undoubtedly existed No hemoglobinuria was present after the fourth hospital day Obvious hemoglobinemia was observed on admission and on several occasions during the

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in saline at room temperature The hemolysin was active only in the presence of complement since inactivation of normal serum at 56 C prevented the hemolysis which occurred with fresh unheated serum

4 *Heterophile Antibody* On admission the heterophile agglutination was positive in a dilution of 1 1024 and remained at that level 10 days after admission on January 19 On February 10 it had fallen to 1 128 at which level it persisted throughout his stay in this hospital Unfortunately it was not possible to absorb the serum with guinea pig kidney or ox cells to determine the variety of heterophile antibody [11]

TABLE 5 — *Absorption of H[illegible] (March 12 1945)*

Table Number	RBC—[illegible] in saline 0.5	Serum—fresh 0.5	Serum—heated 5 min 56 C 0.5	Treatment of mixtures	Hemolysis
1	C	P		Chilled 20 min at 4–6 C, incubated 1 hr at 37.5 C	+
2	C	P		Chilled to 4–6 C centrifuged serum and cells separated at 4–6 C = P and C	0
3	P fresh	P		Chilled 20 min at 4–6 C, incubated 1 hr at 37.5 C	0
4	C_2 (Washed 3 times in saline 25 C)	C		Incubated 1 hr at 37.5 C	+
5	C fresh	P_2		Chilled 20 min at 4–6 C, incubated 1 hr at 37.5 C	0
6	C_2 (Washed 3 times in saline)		C	Incubated 1 hr at 37.5 C	0

P = Patient C = Normal Control red cells washed three times in physiological saline at room temperature Both blood samples were Group A

5 *Blood Grouping and Transfusion Therapy* Upon admission blood grouping with rabbit immune sera and washed red cells of the patient at 37 C indicated that the patient belonged to blood group O The same group was recorded on his Army Identification tag Later it was demonstrated both by us and the North East London Blood Supply Depot that the patient was in reality blood group A as demonstrated by high titer grouping serums No studies were made to determine the possible subgroups A_1 and A

In spite of hemagglutination demonstrated at room temperature and at 4–6 C it was evident that transfusions were necessary to combat the rapid fall of the hemoglobin Since the initial blood group was considered to be group O 1500 cc of group O blood warmed to body temperature was given on the evening of

first four days of hospitalization Subsequent observations of the plasma showed moderate hemoglobinemia above the level of controls until January 16 Quantitative results are not reported because of difficulty in preparing a proper benzidine reagent [7] An attempt was made to test the effect of chilling by placing the patient s arm in ice water on March 13 The experiment had to be terminated in fifteen min

TABLE 3 —*Donath Landsteiner Test*

Tube number	RBC—5% suspension in saline 0.5 cc	Serum—fresh 0 5 cc	Serum†—heated 5 min at 56 0.5 cc	Degree of hemolysis	Treatment of mixtures
1	P*	P		o	Incubated 1 hr at 37 5 C
2	C*	P		o	
3	P	C		o	
4	C	C		o	
5	P	P		2+	Chilled 20 min at 4 6 C incubated 1 hr at 37 5 C
6	C	P		2+	
7	P	C		o	
8	C	C		o	
9	P		P	o	Chilled 20 min at 4–6 C incubated 1 hr at 37 5 C
10	C		P	o	
11	P		C	o	
12	C		C	o	

* P = Patient C = Normal Control red cells washed three times in physiologic saline at room temperature Both blood samples Group A

† *Guinea pig serum was not available as a source of complement*

TABLE 4 —*Observations Following Immersion of Right Arm in Ice Water (March 12 1945)*

	Before test	Right arm immersed 10 min		Right arm blood after chilling 15 m
		Right arm blood	Left arm blood	
Hemoglobin Gm %	13 28	12 96	12 64	12 16
RBC millions	4 05	4 0-	3 88	3 74
Hematocrit %	37	37	37	35
Plasma Hgb mg per 100 cc	48	58	59	70
Reticulocytes %	2 2	2 2	2 4	2 0
Urine hemoglobin	Neg			Neg
Urobilinogen	1 40			1 40

utes because the patient fainted No hemoglobinuria developed and the changes in the red blood cell count and level of free plasma hemoglobin were equivocal (table 4) The high levels for plasma hemoglobin in all control samples were considered to result from the benzidine reagent as mentioned above precautions were taken to prevent hemolysis in obtaining the blood samples

As shown in table 5 the hemolysin was absorbed from the patient s serum at 4–6 C by normal group A red blood corpuscles and was not removed by three washings

Subsequent Cou se an I Present Condition The patient was evacuated to a hospital in the United States as an ambulatory pati nt on March 12 1945 Th re in April the heterophile t st was still positive (tit r unavailable) and s rum phosphorus cal ium and alkaline phosphatase determinations were normal as was an electrocardiogram Roentgenograms showed widespread slight o teoporosis of the tibiae fibulae lumbar vertebra and skull Biopsy of a lymph node from the right inguinal region was reported as showing subacute inflammation not inconsist nt with inf ctious mononucleosis He was transf rred to another hospital in December 1945 when he developed a fissure of the rectum and hemorrhoids and was operated upon uneventfully At this time the s dimentation rat was 32 millimeters per hour whole blood chlorides 479 milligrams per cent CO_2 combining po er 61 volumes per cent serum phosphorus 3 8 milligrams per cent serum calcium 11 3 milligrams per cent and urea clearance test was 72 per cent of normal Se rnal puncture was negative and a modified Donath Landsteiner test was now negati e During this entire period he remained weak unders eight developed pains in his l gs of increasing s verity clubbing of the toes was obs rved at that time with an apparent increas in the clubbing of the fingers He was discharged from the Army in January 1946

On January 21 1947 he entered a Vet rans Administration Hospital for further study where h remained until March 7 His complaints were persist nt aching in the legs a pressure sense in the rectum with f equent mucoid def cations listlessn ss n rvousness and chronic fatigue On physical examination he appeared som what agitated th re was audible hyperperistalsis and tenderness upon palpation of the rectum and prostate clubbing of the fingers and toes cold clammy hands and feet and marked adenopathy in the inguinal femoral and posterior cervical regions

Extensive laboratory studies ere made at this time They are sho n in table 1 In essence all hematologic studies were then within normal limits Six s rologic tests for syphilis were carried out and were negative (Kahn Kolmer Kline Eagle Hinton Mazzini) Stool examination showed mucus but no blood parasites or ova Sigmoidoscopy was normal

Roentgenograms of the chest including bronchograms were normal The hands and feet and long bones showed clubbing of the terminal phalanges of all fingers and toes and there was expansion of the carpal and metacarpal bones with coarseness and trabeculations and thickening of the cortex The skull had a som what granular appearance A barium enema revealed a spast c colon

Discussion

Did this patient have infectious mononucleosis? The heterophile antibody de creased coincident with the decrease in serum hemagglutinins but at all times it was positive in much higher dilution than was the hemagglutinin Belk [14] in studying a patient convalescent from infectious mononucleosis found not only heterophile agglutinins and hemolysins against sheep horse rabbit and pig cells but also autoagglutinins which were active below 10 Centrigrade but not at 37 He suggested that a nonspecific stimulus in this disease might result in a widespread production of antibodies In a study of cold agglutinins Favour[15] found them present in four of ten cases of infectious mononucleosis with a maximum titer of 1 180 Springyarn et al [16] have demonstrated them in seven cases of this disease In their search for cold hemagglutinins in various disease states Finland and his associates[1] found none in the three cases of infectious mononucleosis which they investigated Davidsohn[17] found the titer of isoagglutinins normal in 44 cases of infec iou mononucleosis

The morphologic white blood cell picture of hemolytic anemia is usually de scribed as an absolute and relative polymorphonuclear leukocytosis An absolute and relative lymphocytosis as seen in our patient is unusual and the presence of atypical lymphocytes characteristic of those found in infectious mononucleosis is

Many of the test were generously made by the Blood L boratory of the Pratt Diagnostic Hospital Boston

January 10 There was no reaction The Rh blood group could not be determined at this time since anti Rh serum was not available

Transfusions were continued and a total of 7500 cubic centimeters of blood was given between January 10 and 21 * The patient occasionally complained of pain in the right and left upper quadrants following transfusion but no evidence of increased hemolysis could be demonstrated as having occurred Only group O blood without Rh determination had been given up until January 24 At that time anti Rh serum became available and a mixture of Rh negative and positive cells was demonstrated in the patient s blood Rather than speculate which were the patient s cells and which the donor s in view of the 7500 cubic centimeters of undetermined Rh blood which had been given concentrated Rh negative group A cells from 2000 cc of whole blood were suspended in physiologic salt solution and administered at room temperature on January 24 A transient slight pyrogenic reaction followed No further transfusions were given prior to evacuation to the United States

6 *Urinary Findings* The admission urine specimen January 9 was port wine in color showed 3 plus albumin a 4 plus reaction for hemoglobin and a 1 plus bile test Tests for sugar acetone porphyrin indican alkapton and hemosiderin were negative *Urobilinogen and urobilin were not determined on this specimen* Numerous granular casts were present in the centrifuged specimens but no white blood cells red blood cells or blood cell casts On the second hospital day the urine was dark but not red It contained a 2 plus albumin 2 plus bile but the test for hemoglobin was negative and the sediment was negative Spectroscopic examination of the specimen by the North East London Blood Supply Depot Luton England showed an increase in urobilinogen urobilin and bile but no evidence of porphyrins On the fourth hospital day the morning specimen showed a 4 plus reaction to benzidine an afternoon specimen was free of hemoglobin and no specimen thereafter contained either hemoglobin or bile Urine urobilinogen was positive in 1 40 dilution on the fifth and eighth hospital days and remained positive in 1 80 to 1 160 dilution until January 24 when it became entirely normal Concentration tests and fractional phenosulphonphthalein excretion tests were normal

7 *Miscellaneous Tests* In addition to the laboratory examinations already discussed numerous other tests were carried out and are shown in table 1 All including chest roentgenograms on admission were normal or unrevealing as to the nature of the hemolytic process

Course in Hospital During the first two weeks the patient was critically ill but throughout this period as well as later was active and loathe to stay in bed By January 22 the anemia had ceased to progress and from then on steady improvement occurred A peak reticulocytosis of 9 8 per cent was found on February 10 During the period of marked anemia the patient exhibited a low grade fever reaching 101 F on two occasions As the red blood cell level was restored the temperature returned to normal and after February 8 never exceeded 99 Fluid intake and output were satisfactory The patient gained 5 pounds during the two months hospitalization

*All blood was generously supplied by the N E London Blood Supply Depot Luton England

Summary and Conclusions

A case is reported of a young man with acute hemolytic anemia and hemoglobinuria who presented an initial blood picture consistent with infectious mononucleosis associated with a heterophile agglutination test positive in high dilution auto-hemagglutinins active in the cold, at room temperature and at 37 Centigrade a hemolysin active at 37 C after chilling requiring the presence of a thermolabile component of serum for hemolysis a positive Donath Landsteiner test but no evidence of syphilis In addition there was clubbing of the digits with certain other roentgenologic changes in the bones absence of any other etiologic factors known to be concerned with such anemia uneventful improvement under massive transfusion therapy with apparent recovery from his hematologic disorder when studied two years later

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even more unusual Dameshek[4] has reported an instance of hemolytic anemia in a patient with infectious mononucleosis who had also received sulfadiazine A potent iso and autohemagglutinin especially active at ice box and room temperatures was present in the serum He felt that the drug played an important role in the production of the anemia With this exception no instances of hemolytic anemia occurring in infectious mononucleosis have come to our attention In their review of hemolytic anemias Dameshek and Schwartz[1] cited none In his monograph on infectious mononucleosis Bernstein[13] stated that anemia of any appreciable degree do s not appear unless associated with some complicating feature such as hemorrhage of dietary deficiency

The evidence in this case suggests the possible diagnosis of infectious mononucleosis although it cannot be proved It was not possible to classify the heterophile antibody by absorption studies [11] Although there was a history of an upper respiratory infection two weeks before the first hospital admission there was insufficient evidence to establish a diagnosis of atypical virus pneumonia Since no other etiologic factor for his hemolytic anemia was evident a causal connection between his anemia and the development of abnormal serum antibodies is suggested possibly related to a disease resembling infectious mononucleosis

Another feature of interest is the positive Donath Landsteiner reaction Such reactions usually have been found in cases of paroxysmal hemoglobinuria associated with syphilis [9] although a few instances of its occurrence in hemolytic syndromes in the absence of syphilis have been reported In our patient there was no evidence of syphilis Stats and Wasserman [19] who estimated that 92 per cent of cases showing a positive Donath Landsteiner reaction have an associated syphilis were of the opinion that fundamentally different antibodies are responsible for cold hemagglutinins and a positive Donath Landsteiner reaction They found but one case in the literature in which both have been reported [0] and refer to one furth r case with a positive Donath Landsteiner reaction in which cold hemagglutination at from 0 to 3 Centigrade was observed [21]

A relationship between the clubbing of the digits and the blood changes is unlikely The presence of cold hemagglutinins and the occurrence of hemolytic manifestations have been described in patients with peripheral vascular disease especially of the vasospastic type such as Raynaud s disease [19] Peripheral osteoarthropy is generally considered to be related to abnormal circulation to the bone but the association of clubbing with peripheral vascular disease is rare In the present instance the clubbing preceded the acute hemolytic crisis and progressed after the hematologic abnormalities had disappeared and the hemagglutinins had diminished to a very low titer This patient showed no evidence of peripheral vascular disease other than a tendency toward moderately cold and cyanotic hands and feet with hyperhidrosis of the palms developing after his acute hemolytic episode It is hardly tenable to relate the association of the bone changes to the hemolytic anemia or an illness resembling infectious mononucleosis unless the hypothesis is advanced that there was an underlying circulatory dystrophy with a chronic but minimal production of hemagglutinins which gave rise to a hemolytic crisis when the concentration of these antibodies was increased by the acute disease process

THE MECHANISM OF TRANSPLACENTAL ISOIMMUNIZATION

By PHILIP LEVINE M D

THE PATHOGENESIS of erythroblastosis occurs in two steps (1) isoimmunization of the mother by fetal red blood cells and/or tissue cells (2) intrauterine action of maternal isoantibodies on fetal red blood cells In any event it has been assumed that there is transplacental transfer first of antigenic fetal material into the mother s circulation followed by transfer of maternal antibodies in the reverse direction [1] [2] While maternal antibodies (diphtheria antitoxin pertussis antibodies normal isoagglutinins etc) are known to find their way into the fetal circulation the phenomenon of isoimmunization of the mother either by soluble or formed fetal elements such as red blood cells was mentioned only rarely

In 1905 Dienst[3] suggested that eclampsia may result from the release of incompatible fetal blood through gross defects in the placenta Somewhat later the same idea was revived by Ottenberg[4] who mentioned isoimmunization of the mother by fetal blood The action of the maternal antibodies on the fetal red blood cells was assumed to be the cause of both icterus neonatorum and icterus gravis Hirszfeld[5] wrote at length on this subject in terms of the factors A and B but at no time in his numerous papers was mention made of iso mmunization of the mother by a dominant factor in fetal blood The only homospecifi matings mentioned by Hirszfeld were those in which the blood groups of the mother and the infant were identical As pointed out by Levine [6] the combination of a group A mother and a group O child was classified by Hirszfeld as a heterospecific pregnancy

In 1936 Jonsson[7] demonstrated isoimmunization of the mother by the dominant properties A and B in fetal blood as indicated by the presence of specific hemolysins but no attempt was made to correlate this observation with fetal neonatal morbidity More recently Darrow[8] anticipated the theory of isoimmunization of the mother by fetal elements but no evidence was produced aside from a demonstration of serologic difference of fetal and adult hemoglobin [9] It is significant that none of these workers dealt with specific antigenic factors of red blood cells other than A or B

In the light of our present knowledge regarding secretor and nonsecretor types of the A and B substances there is no mental hazard in accepting the view that the mother may be immunized by water soluble fetal products Historically it is of interest that in their 1939 paper in which a new blood factor was described but not named Levine and Stetson[10] refer to transplacental transfer of an immunizing property in the blood and/or tissues of the fetus which must have been inherited from the father Accordingly it was suspected as early as 1939 that the new blood factor later identified as Rh was limited to red blood cells or perhaps tissue cells and could be classified as belonging to the nonsecretor type This was subsequently confirmed by Levine and Katzin who failed to demonstrate specific inhibition of anti Rh agglutination by saliva or seminal fluid (table 1)

Negative results were also obtained in tests with sperm cells of Rh+ individuals and it was suspected that the Rh factor may be limited to red blood cells [11] The demonstration by Witebsky and Mohn[1] of minute amounts of Rh inhibiting activity in amniotic fluid can have no bearing on the mechanism of transplacental transfer since the erythroblastotic infant belongs to the nonsecretor type It must

From the Rh Blood Testing Laboratory Ortho Rese rch Foundatio Raritan New Jersey

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transfer in one or another form of fetal red blood cells into the maternal circulation Javert[13] suggested that fetal red blood cells may enter the maternal circulation via placental thrombi in which he claims to have observed fetal red blood cells This view could not be confirmed by Potter[15] in a study of 60 placentae which had thrombus like lesions Burnham[16] claimed that lack of or improper utilization of vitamin C produces placental lesions which would permit leakage of fetal red cells Because of lack of evidence this view is generally not accepted

In the first studies which demonstrated the role of the Rh factor in the pathogenesis of erythroblastosis fetalis no attempt was made to describe the mechanism by which the fetal red blood cells a large formed element traverse the placenta In the interval of 1941-1943 a concept was gradually developed which seemed to be compatible with the clinical and serologic findings [17]

It is assumed that in every normal pregnancy minute quantities of fetal red blood cells in one form or another, find their way into the maternal circulation in sufficient quantity to induce isoimmunization According to this view it is not necessary to assume the existence of gross placental lesions which would have to recur with each successive pregnancy in which the fetus is Rh+ Another consideration is the fact discussed below that only minute quantities of blood may suffice to immunize The existence of gross placental lesions can also be excluded since the course of the pregnancy and the delivery in the vast majority of immunized mothers is entirely uneventful Toxic symptoms are apt to occur only in some of the much smaller group of mothers of infants suffering from fetal hydrops and it is probable that these symptoms in the mother cannot be attributed to the effects of gross leakage

From an immunologic viewpoint it is safe to assume that minute quantities of fetal blood may suffice to induce isoimmunization There are numerous examples in serologic literature to demonstrate that very small doses of soluble proteins toxins suspensions of bacteria or red blood cells administered to the experimental animal will result in antibody production Perhaps the most outstanding example is sensitization of the guinea pig to anaphylaxis by the injection of e g 10^{-6} cc of horse serum (ca 7×10^{-8} grams of protein)

In order to accumulate additional evidence with red blood cells as an antigen the author injected a series of rabbits daily with 2 cc of a 1 5000 suspension of citrated human blood After seven injections there was a slight to moderate increase in the activity of the sera for human blood The antibody response was more striking after a second series of injections (table 2)

It is of interest that one of the animals was comparatively resistant to the injections and that one of the remaining five animals produced anti M agglutinins as well as species-specific antibodies Measured in terms of sediment the total volumes employed for the seven and fourteen injections were respectively 0 0014 and 0 0028 cc The corresponding values for an adult of 120 lbs (60 kg) are 0 0336 and 0 0672 cc respectively

It is true that the above mentioned experiment dealt with heteroimmunization and it seems desirable to determine whether or not the same principle applies also to isoimmunization of rabbits with minute quantities of rabbit blood of varying

be pointed out that amniotic fluid contains large quantities of soluble A and B substances in contrast to minute quantities of Rh soluble material

The absence of the Rh factor from body fluids was anticipated because the main clinical and pathologic features of the disease are referrable to blood destruction [2] If the Rh factor were present in body fluids its mode of entry into the maternal circulation would present no difficulty Were this the case the maternal anti Rh antibodies after passage across the placental barrier would be so completely neutralized by the excess of water soluble antigenic substances that the red cells of the fetus would be spared Whether or not this state of affairs actually does occur in incompatible matings with a fetus of group A or B secretor type is not yet

Table 1 —Specific Inhibition of Anti A Anti B and Anti Rh_0 (Anti D) by Saliva

Saliva donor	Group	I Serum Group O Incubated with saliva Tested with blood suspension of group		II Rh serum (Anti D) Incubated with saliva	Rh react
		A	B	Tested with blood O Rh+	
1	A	o	++	+±	+
2	A	o	++	+±	+
3	A	o	++	+±	o
4	A	o	++	++	+
5	A†	++	++	++	+
6	B	++	o	++	+
7	B	++	o	++	+
8	B	+±	o	++	+
9	B	++	o	+±	o
10	B†	++	++	++	+

Before adding blood the test mixture consisted of 0 2 cc saliva and 0 2 cc serum dilution The final dilution of the group O serum was 1 16 that of the anti Rh serum was 1 8 Readings in I were made after the test stood two hours at room temperature in II the readings were made after the test stood for one hour at 37 C

* Modified after Levine and Katzin [11]

† Non secretor

definitely established As pointed out by Levine [6] such a mechanism may perhaps be held responsible for a certain number of early fetal deaths and perhaps stillbirths due to causes other than the hemolytic process

Using an indirect method Boorman and Dodd[13] claim to have demonstrated Rh substances in certain tissue cells but their findings are still to be confirmed Possibly their observations if confirmed may have some etiologic relationship to the group of cases of erythroblastosis fetalis characterized by severe jaundice toxemia and the absence of severe grades of blood destruction Judging by analogy with the A and B substances one may assume that brain cells are entirely lacking in the Rh substances so that an etiologic relationship to kernicterus on the basis of a specific reaction with antibodies for Rh and the corresponding substance in brain cells can be excluded

There is at present no suitable explanation for the mechanism of transplacental

begin until after midpregnancy when certain structural changes occur which are favorable for the transfer into maternal sinuses of minute amounts of one form or another of fetal red blood cells [17] As the placenta grows the blood vessels in the villi at first centrally located become larger and come to lie adjacent to the maternal sinuses During this period the Langhans cells degenerate and only a thin membrane and one layer of syncytial cells separate the fetal and maternal circulations The gradual thinning of the barrier is readily demonstrated in a comparative study of histologic sections of normal placentae of varying periods of development The retention of Langhans cells in the placentae of erythroblastotic infants does not invalidate the view presented since these changes may be considered as secondary responses to the hemolytic process

At the same time there is an ever increasing surface area of fetal villi in contact with maternal sinuses It has been calculated by Dodds[25] and Dees Mattingly[26] that in the term placenta there are from 70–120 square feet of fetal villi exposed to maternal sinuses and the total length of these villi if laid end to end would measure 11 4 miles Furthermore one fourth or more of the fetal blood is outside of the fetus and in close contact with the maternal circulation It is known that the circulation in the maternal sinuses is very sluggish and that the pressure is greater in the fetal circulation Accordingly there is ample opportunity even under physiologic conditions for the escape into the maternal sinuses of a minute number of red blood cells in one or another form One may well speculate that the pressure in the fetal villi is not constant and may be increased as a result of fetal movements which generally become active in the fifth month

The mechanism of isoimmunization described is compatible with the clinical observation that once an Rh− mother delivers an erythroblastotic infant the condition is apt to recur in all succeeding pregnancies in which the fetus is Rh+ Apparently the isoimmunization is renewed even if subsequent pregnancies are spaced at intervals which are sufficiently long for the complete disappearance of antibodies residual from the preceding pregnancy Thus the specific form of the anamnestic reaction is called into play in each successive pregnancy and the determining factor is the presence of Rh+ fetal red blood cells in the maternal circulation during the course of the pregnancy

Transplacental isoimmunization by the Rh factor depends on (1) a combination of an Rh− mother and Rh+ fetus and (2) genetic capacity to respond to the antigenic stimulus It is assumed that the mother whose first Rh+ infant is affected produces antibodies with greater ease than the mother who has several normal Rh+ infants prior to the first affected infant These two facts serve to determine the comparatively low incidence of erythroblastosis in spite of the fact that there are 13 per cent incompatible matings (85 per cent × 15 per cent = 13 per cent) More than 50 per cent of these Rh+ fathers (about 58 per cent) are heterozygous and half of their offspring will be Rh− like the mother Another factor tending to reduce the incidence of erythroblastosis fetalis is the current tendency to small families It is probable that every Rh− mother would produce an erythroblastotic infant provided that there were a sufficient number of pregnancies

The same genetic factors determining the capacity to produce antibodies are

antigenic constitution However there is already indirect evidence obtained from recent experiments on isoimmunization of Rh− donors with small quantities of Rh+blood This applies especially for the increase of antibodies already formed From a practical viewpoint, one must consider that these experiments cannot simulate the conditions existing in pregnancy which are most favorable for anti body production i e slow administration of the antigen over a long period Certainly these conditions cannot be satisfied in either abortions ectopic preg nancies nor in the process of parturition

By and large the effects of isoimmunization by the Rh factor are exerted on the fully, or almost fully developed fetus or the newborn infant Intra uterine fetal death in the seventh or eighth month is never observed in the first born unless the mother has been immunized by previous transfusions of Rh+ blood With increas ing degrees of isoimmunization fetal death may occur but scarcely before the sixth or seventh month Remarkably enough the presence of potent antibodies residual from the preceding pregnancy does not interfere with the process of fertilization implantation nor with the subsequent development and growth of the fertilized

TABLE 2 —*Immunization of Rabbits with Minute Quantities of Human Blood (Group O)*

Time of Test	Agglutination Titers of Five Immunized Rabbit Bloods Tested with Blood Group O				
	1	2	3	4	5
Before injections	5	10	5	5	5
After 7 injections	15	100	25	25	10
After 14 injections	100	300	25	600	100

After Levine [17]

ovum even though red blood cells are already in the process of formation in the fourth week [9] The Rh factor has been demonstrated in the blood of a 48 mm fetus by Stratton [1] and a 17 cm fetus by Bornstein Diamond [23] demonstrated the Rh factor in the blood of three fetuses of 3 month development while Potter[15] found the factor present in fifteen out of seventeen fetuses weighing between 8 and 200 grams There is reason to suspect that the more fundamental property of anti genicity and capacity to unite with antibodies may be inherent in the earlier fetus or even in the embryologic forerunners of red blood cells

Although immunized Rh− mothers have a somewhat higher incidence of abor tions and miscarriages [2] [24] there is little or no statistical proof to indicate that early fetal death is brought about by the action of passively transferred maternal antibodies While metabolites are transferred early in the course of development there is no proof that the early fetus receives maternal antibodies Certainly the fetus does not require antibodies at this early stage of its development In any event this subject and its possible relationship to early fetal death merits further investigation Whether or not an early abortion followed by a curettage results in the transfer of fetal blood into the maternal circulation with subsequent isoim munization[15] will be referred to below

According to the author s concept isoimmunization by the Rh factor does not

the serum failed to sensitize Rh+ cells so that the precipitin reaction of Coombs Mourant and Race[3] was completely negative In view of her history the patient s serum was titrated and as was to be expected the existence of a prozone in the lower dilutions was excluded [23] Assuming a two or three months preparatory period one may speculate that fetal red cells began their passage between the twenty sixth and thirtieth week of the pregnancy Since there were six transfusions of which probably five were with Rh+ blood the patient must be considered as relatively resistant to the production of antibodies

Curiously enough there are remarkably few cases in which there are sufficient data useful for this analysis As a rule these patients do not submit to the test until late in the course of the pregnancy when antibodies are already demon strable and their origin whether residual or newly formed cannot be determined However indirect evidence may be obtained in an analysis of the interval between the last transfusion and the first pregnancy with an Rh positive fetus In a combined series of 52 cases of erythroblastosis fetalis in the first full term Rh+ infants the average interval was six years and as mentioned above it is safe to assume that (1) the antibodies which may have been present earlier will have disappeared in the six years interval and (2) that the appearance of antibodies responsible for erythroblastosis fetalis was the result of intra uterine passage of fetal blood *

Of the 52 cases 10 had previous histories of abortions or ectopic pregnancies and 9 of the 10 required blood transfusions The average interval between the trans fusion and the first pregnancy resulting in an erythroblastotic infant was 3 7 years The shorter interval does not indicate that the abortions resulted in isoimmuniza tion In women of child bearing age one cannot expect to find long intervals between pregnancies Excluding this series the interval in the remaining 42 cases between the transfusions and the first pregnancy was 6 4 years

In 2 cases a history of intramuscular injection of blood could be elicited at in tervals of eleven and seventeen years respectively prior to the delivery of the erythroblastotic infant in the first pregnancy The indications for the administra tion of blood were prophylaxis against measles and poliomyelitis Each of these 2 patients denied having previous pregnancies and/or abortions

Finally there is the group of twelve instances of erythroblastosis fetalis in the first born in which there is neither a history of transfusion or abortions An addi tional 4 patients gave a history of early abortion However this can play no es sential role in initiating transplacental isoimmunization which requires prolonged and slow administration of Rh positive fetal blood It was in this group of cases that the suggestion was first made by Levine that these patients may have had intramuscular injections of blood many years previously In the event that this antigenic stimulus can be excluded these Rh− women can be considered as the group most susceptible to isoimmunization Unfortunately there is as yet no known procedure which will differentiate this group from the group which is more resistant to isoimmunization

Possibly fetal red blood cells escape into the maternal circulation in the course

*This includes a second s-ries of cases to be p blished by Levine and Ros-nfield [24]

also operative in isoimmunization of voluntary donors by administration of Rh+ blood Thus, Diamond and Wiener report successful isoimmunization in somewhat less than 50 per cent of the Rh− donors Of 200 Rh− random individuals who were transfused indiscriminately, about 46 per cent produced antibodies for Rh It is most significant that the antigenic response was independent of the number of transfusions but determined rather by certain genetic properties In contrast to the patient reported by Dacie and Mollison[28] who produced antibodies soon after the first transfusion is an Rh− patient studied by Levine who produced antibodies after a series of numerous transfusions [9]

As pointed out by Levine, the occurrence of erythroblastosis fetalis in the first born with or without previous transfusions has considerable bearing on the mechanisms of transplacental isoimmunization In both groups of cases there is a selection of those Rh− women who produce antibodies readily but of the two the nontransfused group is obviously the more sensitive to the administration of Rh+ blood The objection has been raised that many of these women had one or more abortions probably premarital which are not obvious from the usual history This raises the question whether or not fetal red blood cells may be carried over into the maternal circulation as a result of either a spontaneous abortion or the subsequent curettage However this is not likely particularly if there is considerable uterine bleeding which would tend to carry with it the fetal blood In any event an early pregnancy cannot supply the conditions favorable for isoimmunization i e slow administration of the antigen over a long period In a number of cases studied by the writer a history of abortion was denied A more important consideration is the likelihood that many of these women had previously received an intramuscular injection of presumably Rh+ blood [31] In the pre vitamin K days *the intramuscular injection of blood was a common practice and* more recently such histories were obtained particularly if the indication was prophylaxis for either measles or poliomyelitis Under such conditions the erythroblastosis fetalis in the first born is classified under the transfused group

Erythroblastosis fetalis in the first born is a more common event in the transfused group and in many of these there is a long interval between the last transfusion and the first pregnancy It is not likely that the antibodies produced as a result of one or more transfusions will be demonstrable for more than several years but certainly not for five years at least in the vast majority of the cases Accordingly it may be assumed that in many of these cases no antibodies are present when the first pregnancy is started The subsequent appearance of antibodies during the course of the pregnancy provides proof that fetal Rh+ blood cells must have passed the placental barrier in sufficient quantity to exert its antigenic effect

The following case of erythroblastosis fetalis in the first born referred by Dr Regina Beck of Richmond Virginia is cited because antibodies which were not demonstrable either early in the course of the pregnancy i e the twelfth week or in the thirty *third week were found in a specimen drawn in the thirty-eighth* week (titer = 1 64) This Rh− patient was transfused six times in 1941 for gastroenteritis five years prior to her first pregnancy It is significant that in the specimen drawn seven weeks prior to the expected date of confinement (July 17 1947)

These findings raise the question as to the nature of the globulin antibody produced by the immunized mother One may speculate that the antibodies produced represent an ever changing configuration of the immune globulin without losing its characteristic specificity It is not excluded that at times an antibody may be produced which has little or no capacity to pass the placental barrier Possibly this may serve to explain the rare instances of entirely normal or mildly affected Rh+ infants delivered by intensively immunized Rh− women whose previous pregnancies resulted in severely affected infants (Chown[25])

Addendum

Claims were made recently that physiologic breaks in the villi could be demonstrated either by means of serial sections (Naeslund and Aren[29]) or by the presence of nucleated red cells of the erythroblastotic fetus in intervillous spaces (Kline[40]) These observations require confirmation because of the inherent difficulties in providing histologic proof of physiologic breaks in the continuity of blood vessels of such delicate tissue as the placenta

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of the second stage of labor when the placenta begins to separate * Perhaps this serves to explain the increase of antibody content occasionally observed in the postpartum period However, parturition by itself can play no essential role in the group of cases discussed above and by the same token its importance in the mechanism of isoimmunization in general can be excluded

As indicated above there is no final proof for the concept that fetal blood cells find their way into the maternal circulation in every normal pregnancy However this view seems to be compatible with serologic, clinical and pathologic features of erythroblastosis fetalis Most workers hesitate to accept the theory of transplacental isoimmunization probably because of the traditional concept that the placenta presents a barrier which is absolutely impermeable to formed elements It would indeed be quite unique if nature repeatedly provided an absolutely perfect organ which in the course of its short life of 40 weeks attains a surface area of 70–120 *square feet required for the nourishment of the fetus* This would be all the more remarkable because of the rapid proliferation of the trophoblast which is endowed with invasive properties similar to that of the malignant cell

A discussion of the mechanism of transplacental isoimmunization cannot be complete without reference to the passage of maternal antibodies into the fetal circulation Recent attempts to associate prolonged intra uterine action on the part of blocking antibodies with hemolysis and rapid action of agglutinins only at delivery with severe jaundice and toxicity have been shown to be premature [24] Although there are striking differences between agglutinins and blocking anti *bodies demonstrable in vitro these differences lose their significance* because in vivo both react with fetal blood suspended in a medium of plasma In any event blocking antibodies in contrast to anti Rh agglutinins are frequently demonstrable in the infant s circulation

The study of affected infants before and after a more or less complete replacement transfusion reveals the significant fact that appreciable quantities of blocking antibodies can still be demonstrated at the end of the replacement transfusion when the vast majority of the blood is not coated Presumably large quantities of blocking antibodies are stored in the tissue spaces and hence the continued blood de *struction of any residual Rh+ fetal blood in the neonatal period* Certainly there seems to be little or no indication for the use of Rh+ blood in transfusing affected infants of Rh− mothers

With an antigenic stimulus acting over a period of several months in any one pregnancy and its renewal in the following pregnancies with Rh+ fetuses it is not surprising that more than one variety of antibody is produced In a sense many Rh− women are subjected to hyperimmunization More recently a third variety of antibody could be demonstrated in certain blocking sera which are characterized by a distinct prozone in the lower dilutions The antibody responsible for the prozone can be specifically absorbed after contact of the undiluted serum with Rh+ but not with Rh− blood (Levine and Wigod) †

*Should the Rh factor be found in syncytial cells their liberation in the postpartum period may also serve to immunize

†Cf Hill and Haberman [27]

ERYTHROBLASTOSIS FETALIS IN NEGROID INFANTS

By A S Wiener M D and I B Wexler M D

THE INCIDENCE of erythroblastosis among Caucasoids as given by different authors varies from 1 in 400 to 1 in 150 births [1] [2] Despite the tremendous increase in interest in the disease since Levine et al [3] and Burnham[4] demonstrated the role of the Rh factor of Landsteiner and Wiener[5] [6] in its pathogenesis no reports have come to our attention concerning the appearance of the disease in Negroid infants We have recently encountered 3 such cases having unusual features and these are the subject of this report

Case Reports

Case 1 The patient a male infant was born at the St John s Hospital on October 8 1946 This was the mother s first pregnancy and the entire period of gestation was uncomplicated There was no history of luetic infection and the Wassermann reaction of her blood was negative Delivery was spontaneous at term and the infant cried immediately The only abnormality noted at the delivery was a yellow dis coloration of the vernix and amniotic fluid while the cord was normal The birth weight was 7 pounds 8 ounces

The infant appeared to be normal until the second day of life when moderate icterus of the skin and sclerae was noted There was no enlargement of the liver or spleen and the baby appeared to be otherwise well On the following day however jaundice became more intense and the infant vomited its feedings Laboratory findings at this time were as follows Hemoglobin concentration of the blood 13 grams per 100 cc red blood count 4 5 million per cu mm white blood count 9 500 per cu mm There were 2 normoblasts per 100 white blood cells on the smear The stool was yellow green and gave a negative test or blood Bleeding coagulation and prothombin times were normal The infant s red blood cell fragil ity was normal Both the mother and the baby were found to be Rh positive

The hemoglobin fell slowly for the next few days and on the sixth day of life was found to be 9 5 Gm per 100 cc with a red count of 3 95 million per cu mm One eosinophile and 2 normoblasts were seen on the smear at this time Because of the falling hemoglobin and red count a transfusion of 50 cc of group B Rh positive blood was given On the day following the transfusion the hemoglobin had risen to 11 8 grams per 100 cc with the red count 3 92 million per cu mm This rise was not maintained however and a second transfusion of group B Rh positive blood was given Twelve days later the blood count had again fallen and now showed a hemoglobin of only 7 6 grams per 100 cc and a red count of 2 67 million per cu mm

Because of the obscure etiology of the patient s anemia and the poor response to transfusion therapy the case was referred to us for further study Results of grouping and Rh Hr tests on the father mother and infant were as follows

Blood of	Group	M N Type*	Rh Hr Type†
Father	AB	N	Rh_2
Mother	O	MN	Rh_0
Infant	B	MN	Rh_0

From the Transfusion Division of the Department of Laboratories and the Department of Pediatrics of the Jewish Hospital of Brooklyn

* The types M N are not of clinical significance but are included for the sake of completeness

† For heredity and nomenclature of the Rh Hr types see Wiener [7] [8]

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to the A B or Rh Hr factors A coating test (conglutination technic)[11] done on the baby s cells was negative and tests done on the mother s serum for the presence of abnormal iso-antibodies were negative

Transfusion to combat the anemia was indicated and the type of blood to use presented a problem In view of the possibility that the case might be one of isosensitization against a blood factor as yet undiscovered it was decided to use the mother s washed red cells because her erythrocytes could not contain the hypothetical immunizing factor The child was therefore transfused with th washed red cells obtained from 100 cc of the mother s blood resuspended in saline [12] On the day following transfusion the hemoglobin had risen to 15 grams per 100 cc Two days after birth jaundice was found to be present and the hemoglobin had fallen to 11 grams per 100 cc A blood smear at this time showed that there were 2 6 nucleated red blood cells per 100 white cells Periorbital edema appeared and the transfusion of mother s blood using the washed red cells obtained from 100 cc of blood was repeated Response to this transfusion was good in that the hemoglobin now ros to 15 5 grams per 100 cc and the red count to 5 67 million per cu mm However there still were 216 nucleated red blood cells per 100 white cells on the smear Within two days the nucleated red cell count had fallen to 12 per 100 white blood cells and five days following the second transfusion nucleated red c lls wer no longer present Fragility tests done on the t enty first hospital day were within normal limits Numerous sickling preparations were negative immediately and after twenty four hours Cultures of the nose and throat stool and blood failed to reveal any pathogenic organisms X ray of the skull sho ed no abnormalities The child s clinical course was uneventful She was discharged at the end of the fourth week w ighing 5 pounds 9 ounces with a hemoglobin of 10 grams per 100 cc and a red cell count of 2 85 million per cu mm She had no jaundice at the time of discharge When seen in the out patient department four weeks after discharge she weighed 8 pounds 1 ounce Her hemoglobin had fallen to 9 1 grams per 100 cc and her red count was 2 9 million per cu mm but otherwise she seemed well

Comment

If the Rh factor played the same role in all races as it does in the Caucasian one would expect the incidence of erythroblastosis in the various races to correspond with the frequency of the Rh negative type This expectation has apparently been fulfilled in the Mongolian race since erythroblastosis is extremely rare amongst these peoples [13] Among Negroids with a frequency of Rh negative individuals variously reported as between 5 and 10 per cent one might similarly expect an incidence of erythroblastosis from one third to two thirds as high as in Caucasians As we have already mentioned however erythroblastosis appears to be rare among Negroes indicating that considerations other than the Rh type play an important role in the pathogenesis of the disease It is particularly remarkable that in the 3 cases reported here with the clinical picture of erythroblastosis none showed evidence of Rh sensitization Somewhat similar observations have been made by Zuelzer [14]

Recent observations indicate that the efficiency of sensitization in Rh negative individuals depends in part upon the amount of Rh positive blood inoculated into the body For example Rh negative women who at the first pregnancy have had stillbirths due to Rh sensitization almost always show a history of having received a transfusion or intramuscular injection of blood some time in the past [15] Furthermore experiments done to produce Rh testing sera in male donors have shown that *the great majority of Rh negative individuals are readily sensitized* by properly spaced injections of as little as 2 cc of Rh positive blood [16] On the other hand the observation that isosensitization by pregnancy appears to occur in only 1 out of 25 to 50 Rh negative women may be explained by the fact that during pregnancy or parturition only minute quantities of Rh positive blood enter the maternal

Titrations of the alpha and beta antibodies in the mother s serum showed an anti A titer of 15 units by the agglutination method and also *15 units by the conglutination method the anti B titer was 60 units* by the agglutination method by the conglutination method a titer of 600 units was obtained These results show that the patient s anemia and poor response to transfusion were due to sensitization of the mother (group O) to the infant s erythrocytes (group B) On our recommendation two small transfusions of group O red cells (plasma free) were *then administered after which the infant made an unevent*ful recovery When seen again in the clinic at the age of 7 weeks the infant weighed 9 pounds 5 ounces and had no jaundice Blood count was not done

Case 2 A Negress was referred to us with the following obstetrical history Her first pregnancy in 1941 resulted in the birth of a normal male infant who is living and well The second pregnancy in 1943 also gave rise to a normal male infant Her third pregnancy terminated prematurely (8 months) with the birth of a male infant who became jaundiced at the age of 3 days He was kept at the hospital for three weeks during which time the jaundice subsided He was not transfused during his hospital stay and appeared well on arriving home Shortly thereafter however he was seen to be pale He was transfused immediately but lost weight developed jaundice and died The mother was now pregnant for the fourth time and the problem of the prognosis and treatment for the expected infant was presented to us According to the mother previous tests had shown her to be Rh negative and her husband Rh positive

Results of grouping Rh Hr tests on the father mother and the two surviving sons were *as follows*

Bl od of	G oup	M N Typ	Rh Hr Type
Father	B	M	Rh_1
Mother	O	MN	Rh_1Rh_2
1st son	B	M	Rh_1Rh
2nd son	O	M	Rh_1rh

These results show that the Rh Hr types had nothing to do with the problem and that the previous report of the mother s Rh type was in error Titration of the alpha and beta antibodies in the mother s serum showed an anti A titer of 25 units by the agglutination method and a titer of 20 units by the conglutination method The *anti B titers on the other hand were 100 units by the agglutination* method and 500 units by the conglutination method

These findings support the diagnosis of erythroblastosis as the cause of death of the third infant but with the B factor and not the Rh fa tor as the sensitizing agent It must have been the first child who sensitized the mother while the second escaped because it belonged to group O The prognosis for the expected infant now depends upon its blood group If it belongs to group O (50 per cent chance) it will not be erythroblastotic If however it belongs to group B it will almost certainly ha e the di ease In such cases the prophylactic injection of soluble A and B group substances into the infant by way of the umbilical vessels at the time of birth may erve to ameliorate the disease [10]

Case 3 The patient was a second child female born at term after a short labor Pregnancy was uncomplicated and the Wassermann and Kline reactions of the mother blood were negative The baby weighed 5 pounds 9 ounces at birth and was seen to be lethargic pale and appeared to have difficulty in breathing A bradycardia of 110 beats per minute was pre ent There was no apparent jaundice nor was the amniotic fluid or vernix discolored A blood count done shortly after birth sho ed a hemoglobin concentration of only 7 7 grams per 100 cc with a red cell count of 2 32 million per cu mm a whit cell count of 86 000 per cu mm and 82 nucleated red blood cells per 100 white c lls on the smear The mother s blood was found to be A_1MNRh rh and the baby s blood A_1MRh rh There was therefore no known factor present in the infant s erythrocytes that was *lacking from the mother s and no sensitization was possib e*

By the method of titration used the average normal titer with the agglutination technic is approximately 40 units In nonsensitized individuals the titer by the congl tination technic is lower or at least *not higher than* that by the agglutination technic [9, 10]

to the A B or Rh Hr factors A coating test (conglutination technic)[11] done on the baby s cells was negative and tests done on the mother s serum for the presence of abnormal iso-antibodies were negative

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circulation, and at intervals not necessarily optimal for the stimulation of anti body production Another factor is the constitutional ability or lack of ability to be sensitized [17] The situation is analogous to that existing in allergic disea es[18] or *infections* An overwhelming dose of antigen or pathogenic organism will sensitize or infect all human beings a minute dose will affect only the most susceptible Accordingly if one assumes that in Negroid races the placenta offers a better barrier to the passage of materials from the fetus to the mother this would ac count for the rarity of erythroblastosis among these peoples An alternative pos sibility is that among Negroids the frequency of individuals easy to sensitize is very low

Past investigations have furthermore demonstrated that injections of soluble A and B substances in small amounts of secretions such as saliva may give rise to sensitization to these factors while corresponding doses of red cells containing these substances are inadequate to sensitize [19] Therefore passage of soluble mate rials from fetus to mother may give rise to A and B sensitization under conditions which would be inadequate to cause Rh sensitization because comparable small quantities of red cells would not be antigenic It is therefore significant that 2 of our 3 cases can be explained on the basis of A and B sensitization With regard to the third case no evidence of isosensitization could be obtained and if erythro blastosis is strictly defined as comprising those conditions in the newborn caused by isosensitization of the mother by an antigen in the fetal blood then this case does not satisfy the conditions according to our findings However there are a host of clinical conditions producing jaundice anemia hepato splenomegaly and erythroblastemia in the newborn Cases such as this in which no satisfactory con clusion could be drawn demonstrate that there is still much to be learned in this most interesting field

Summary

Three cases of Negroid infants with clinical signs and symptoms resembling erythroblastosis fetalis were presented In none of the cases was there any evidence of Rh sensitization Two of the cases were apparently due to sensitization of the mother to the B agglutinogen in the third case no serologic incompatibility could be demonstrated The possible significance of these observations was discussed

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OBSERVATIONS ON THE MECHANISM OF HEMOLYSIS IN PAROXYSMAL (COLD) HEMOGLOBINURIA

By A A Siebens M D W H Zinkham M D, and Philip F Wagley M D*

PAROXYSMAL (cold) hemoglobinuria is a condition observed usually in syphilitics and characterized by transient hemoglobinemia and hemoglobinuria following exposure to cold Such episodes of hemolysis may be associated with fever chills headache general malaise, abdominal cramps nausea vomiting hives and Raynaud s phenomena The most common complaint is dark or bloody urine following exposure to cold

As late as 1868 this condition had not been differentiated from the hemoglobinuria of malaria [1] However by the end of the nineteenth century all of the clinical features mentioned above were recognized and the condition established as a clinical entity [2] From 1909 to 1949 occasional observations suggested that factors other than cold might activate this hemolytic system For example van den Bergh[3] and Hannema and Rytma[4] observed that carbon dioxide under specified conditions in vitro might affect the degree of hemolysis Manneberg and Donath[5] reported the same results but also observed that carbon dioxide caused hemolysis in normal human blood Kumagai and Ito[6] reported equivocal results Although Mackenzie could not duplicate van den Bergh s results in vitro he observed attacks of hemoglobinuria in a case following emotional disturbances without exposure to changes in temperature [7] Both van den Bergh and Mackenzie concluded that factors other than cold might activate this system

Some observers state that serum complement must be present during the cold phase of the Donath Landsteiner reaction for the hemolysin to be active [8 10] Other investigators have reported that complement need be present only during the warm phase [11 1] Mackenzie has stated that although complement may not be essential for the first phase of the Donath Landsteiner reaction it is adsorbed during the chilling process when present [1] In some of his experiments the hemolysin apparently was fixed on the erythrocyte in the absence of complement but supplementary observations suggested that the presence of complement during chilling increased the degree of hemolysis occurring subsequently at body temperature Differences in the thermolability have been reported[11] for the hemolysins studied in various cases

In this communication observations are reported in an attempt to re evaluate several of these reported discrepancies Two cases of paroxysmal (cold) hemoglobinuria were studied as described briefly in the appendix of this report The experiments are divided into two groups

1 The first deals with the fixation of the hemolysin and complement on the

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erythrocytes This group of experiments shows that the hemolysin and at least one component of complement are fixed to the red cells in the cold and that some thermolabile component of complement must be present in the warm phase of the Donath Landsteiner reaction for the hemolysin to be effective

2 The second demonstrates that although carbon dioxide under specified conditions in vitro affected the hemolytic system in the serum of one patient it failed to do so in the serum from another patient Furthermore certain substances prevented the hemolytic activity in the serum from one case but failed to do so in the serum from a second case The effectiveness of these inhibitors did not depend on prevention of fixation of hemolysin on the red cell in the cold phase of the Donath Landsteiner reaction

Materials and Methods

Group O erythrocytes were washed three times and resuspended in normal saline in a concentration of 5 per cent by volume Guinea pig serum diluted 1 to 5 with normal saline was employed as complement The complement titer* was recorded as the highest final dilution of serum causing hemolysis in a system containing 0 1 cc aliquots of a 1 5 per cent suspension of sheep cells sensitized with rabbit amboceptor to which serial dilutions of 0 1 cc of serum were added Serum was obtained from blood drawn from the patients into syringes and tubes warmed to body temperature Standard technics were employed for determinations of carbon *dioxide cyanide and sulfanilamide*[14 15] *Hydrogen ion determinations* were made with a glass electrode employing a Beckman meter The Donath Landsteiner test was done according to a standard technic[16] The titer of cold hemolysin in the serum was determined by employing serially diluted serum and a constant amount of complement in the Donath Landsteiner test The anti human serum rabbit serum was prepared and used as described by Coombs et al[17]

Experimental Results

Group I *Experiments on the fixation of hemolysin and complement during the Donath-Landsteiner reaction*

The object of the following experiment was to determine the phase of the Donath Landsteiner reaction during which complement and hemolysin were adsorbed by the erythrocyte

A Experiment on fixation of hemolysin and complement in the cold

Washed normal Group O red blood cells were packed in 0 5 cc aliquots in four tubes (see table 1) To two of the tubes (Nos 1 and 3) 2 cc of serum from Case 1 were added To the other two tubes (Nos 2 and 4) 2 cc of serum from Case 2 were added Then 0 5 cc of saline was added to all tubes Tubes 1 and 2 were kept at 27 C for one hour with frequent mixing Tubes 3 and 4 were chilled at 2 C for thirty minutes and then centrifuged after being packed with ice in large centrifuge metal cups Tubes 1 and 2 were centrifuged at 27 C The clear supernatant was removed quickly from all four tubes

Results and conclusions The titer of complement and hemolysin in the supernatant removed from the cell serum mixtures kept at 27 C were both high However both hemolysin and complement activity disappeared from the serum previously chilled at 2 C in the presence of cells Presumably hemolysin and one or more components

* The term complement activity is used to indicate the hemolytic reaction of serum with sensitized sheep cells as described above Since all components of complement are necessary for the hemolytic reaction with sensitized sheep cells the absence of complement activity is interpreted as indicating the lack of one or more these components

of complement were adsorbed by the red blood cells at the lower temperature but not at the higher one. Such adsorption of hemolysin and complement from the serum of Case 2 caused no significant alteration in the electrophoretic pattern of the serum.[18]

The question then arose as to what would occur if hemolysin and complement were separated and chilled separately in the presence of red blood cells.

B. Experiment on complement and hemolysin activity in the cold phase of the Donath-Landsteiner reaction

Serum from Case 2* was heated at 63 C for three minutes. Two-tenths of a cubic centimeter of this serum caused no hemolysis of 0.2 cc of a 1.5 per cent suspension of sensitized sheep cells (see tube 1 of table 2). The remainder of the serum was divided into 0.5 cc aliquots and, as shown in table 2, was placed in tubes 3 and 5. Five tenths of a cc of unheated serum were placed in two other tubes (see tubes 2

TABLE 1.—*Adsorption of Hemolysin and Complement*

Test tube	Case 1 serum	Case 2 serum	Control packed R B C	Normal saline	Hemolysin in supernatant*	Complement in supernatant†
	cc	cc	cc		titer	titer
1	2.0		0.5	0.5	1/4	1/128
2		2.0	0.5	0.5	1/32	1/32
3	2.0		0.5	0.5	0	0
4		2.0	0.5	0.5	0	0

Procedure: Test tubes 1 and 2 left at room temperature (27 C) for 1 hour, then centrifuged at 27 C and supernatant tested for hemolysin and compl. Test tubes 3 and 4 chilled at 2 C for 30 mins with frequent stirring, then centrifuged in cold and supernatant tested for hemolysin and compl.

* As determined by Donath-Landsteiner tests on serially diluted supernatant.

† As determined by adding 0.1 cc 1.5% sensitized sheep R B C to 0.1 cc portions of serially diluted supernatant.

and 4 of table 2). To the four tubes were added 0.2 cc of a 5 per cent suspension of washed normal Group O red blood cells. One tenth cc of normal saline was added to all four tubes. To tubes 2 and 3 were added 0.2 cc of a 1 to 5 dilution of guinea pig serum. All four tubes were then chilled at 2 C for ten minutes. After the chilling, 0.2 cc of a 1 to 5 dilution of guinea pig serum were added to tubes 4 and 5. All tubes were then incubated at 37 C for 1 hour.

Results and conclusions. As shown in table 2, hemolysis did not occur if cells were chilled in heat inactivated serum unless complement was added *before* chilling. Adding complement to previously heated serum *after* the chilling phase of the Donath-Landsteiner reaction did not cause hemolysis. Apparently complement had to be present during the cold phase of the reaction to cause hemolysis. This was also suggested by rechilling tube 5 of table 2. Such subsequent rechilling and rewarming resulted in almost complete hemolysis. This substantiated the conclusion

* The hemolysin in the serum of Case 1 was found to be more thermolabile than complement when heated at 56 C. Attempts to separate the thermolabile hemolysin and the complement by adsorption on other antigen antibody precipitates failed since both were adsorbed simultaneously. In contrast, the hemolysin in the serum of Case 2 was thermostable, thus allowing inactivation of the complement without destroying the hemolysin.

that hemolysin was present but was active only when chilled in the presence of complement

These conclusions were substantiated by another technic The serum of Case 2 and guinea pig serum were heat inactivated at 57 C for thirty minutes These sera and unheated sera were combined as shown in table 2 A and chilled with cells at 3 C for ten minutes then incubated at 37 C for thirty minutes Small aliquots of cells chilled in the presence of the various combinations of sera shown in table 2 A

TABLE 2 —*Effect of Complement on the Cold Phase of the Donath Landsteiner Reaction*

Test tube	Case 2 serum	Case 2 serum heated at 63 C 3m	Cont R B C	Compl added before chilling	Compl added after chilling	Normal saline	Sensitized sheep R B C (1.5%)	Hemolysis
	cc	cc			cc		cc	
1		0.2					0.2	0
2	0.5		0.2	0.2		0.1		++++
3		0.5	0.2	0.2		0.1		+++
4	0.5		0.2		0.2	0.1		++++
5		0.5	0.2		0.2	0.1		0

Procedure All tubes chilled at 2 C for 10 minutes then incubated at 37 C for one hour

TABLE 2 a —*Demonstration of Adsorption of the Hemolysin on Red Cells by the Agglutination of such Cells (in a Saline Suspension) following the Addition of Anti human Serum Rabbit Serum*

Test tube	Case 2 serum	Case 2 serum heated at 57 C 30 m	Complement	Complement heated at 57 C 30 m	R B C (5 per cent suspension)	Hemolysis*	Coombs serum agglutination†
					c		
1	0.5		0.2		0.3	2+	2+
2		0.5	0.2		0.3	tr	1+
3		0.5		0.2	0.3	0	0
4	0.5			0.2	0.3	1+	2+

* Hemolysis as determined after chilling the suspension 10 minutes and incubating at 37 C for 1 hour

† Coombs test was performed by incubating 0.2 cc of a cell suspension with 0.2 cc Coombs serum and expressing the agglutination as 4+ to 0 on microscopic examination

were removed prior to incubation at 37 C and resuspended in several cc of saline By resuspending the cells in saline immediately after chilling they could be kept indefinitely without lysis (see tubes 3 and 6 of table 3) Two-tenths of a cubic centimeter of such suspensions of red cells were then incubated at 37 C for one hour with Coombs serum [17]

Hemolysis occurred only when the serum of Case 2 or that of the guinea pig had not been previously heated Heating both sera prior to the chilling phase of the Donath Landsteiner test prevented subsequent hemolysis Cells after being chilled in the presence of both hemolysin and intact complement and resuspended in saline were agglutinated by anti human serum rabbit serum [17]

This agglutinability of the cells occurred following their chilling in the presence of the hemolysin when guinea pig serum furnished the only complement it did not occur when both the complement in the patient s and guinea pig sera had been previously heat inactivated These observations suggested the hemolysin was adsorbed only in the presence of intact complement whether the latter was furnished by the human serum or guinea pig serum

Since both the hemolysin and at least the thermolabile components of complement were required in the cold to sensitize red cells the role of complement during the warm phase of the Donath Landsteiner reaction was studied Therefore the following experiment was performed

TABLE 3 —*Effect of Complement on the Warm Phase of the Donath Landsteiner Reaction*

Test tube	Sensitized cont R B C (5%*)		Compl	Compl heated at 63 C 3 min	Normal saline	Hemolysis
	Case 1	Case 2				
	cc	cc	c	c	cc	
1	0 2		0 2			++++
2	0 2			0 2		0
3	0 2				0 2	0
4		0 2	0 2			++++
5		0 2		0 2		0
6		0 2			0 2	0

Procedure Compl heated compl and normal saline added to sensitized cont R B C at room temperature (circum 27 C) All tubes then incubated at 37 C for one hour

* Sensitized cont R B C prepared by chilling cont R B C for 15 mins at 2 C in presence of sera of case 1 and case 2 R B C then washed with normal saline and resuspended in 5% concentration

C Experiment on complement and hemolysin activity in the warm phase of the Donath-Landsteiner reaction

Packed washed normal Group O red cells were chilled at 2 C for fifteen minutes in twice their volume of unheated serum from Case 1 or Case 2 (see table 3) Saline chilled to 4 C was then added and the cells washed while cold They were then resuspended in normal saline in 5 per cent concentration and warmed to 27 C Two tenths of a cubic centimeter of the suspension of red cells previously chilled in the serum of Case 1 were placed in three tubes (tubes 1 2 and 3 of table 3) and equal aliquots of cells previously chilled in the serum of Case 2 were placed in three other tubes (tubes 4 5 and 6) Two-tenths of a cubic centimeter of guinea pig serum diluted 1 to 5 were added to tubes 1 and 4 0 2 cc of guinea pig serum diluted 1 to 5 with saline and inactivated by heating at 63 C for three minutes were added to tubes 2 and 5 and 0 2 cc of saline were added to tubes 3 and 6 All the tubes were then incubated at 37 C for one hour

Results and conclusions Cells that were previously chilled in the presence of fresh serum from both cases then washed and resuspended in saline did not hemolyze on incubation unless intact complement was present also during the warm phase Adding heat inactivated complement or more saline did not cause hemolysis Presumably red cells sensitized in the cold phase by hemolysin and complement could not be hemolyzed in the warm phase unless intact complement was present Thus

some thermolabile component of complement was required during the warm phase of the Donath Landsteiner reaction

Group II Experiments with carbon dioxide and inhibitors of carbonic anhydrase

As previously reported [19] relatively high contents of carbon dioxide under certain conditions in vitro may cause hemolysis of cells suspended in the serum of some cases of paroxysmal (cold) hemoglobinuria Other observers [8] in similar experiments have not obtained these results In a previous communication [19] preliminary observations were made on the effect of carbon dioxide on the Donath Landsteiner reaction of Case 1 as reported in more detail here One possible interpretation for the discrepancy in the reports concerning the effect of carbon dioxide might be that there were possible differences in the temperatures at which the experiments were performed by different observers For example it had been reported[19] that carbon dioxide caused complete hemolysis of Group O cells suspended in the patient s serum (Case 1) when the temperature was 27 C but no hemolysis occurred at 37 C It was conceivable therefore that exposure to relatively high temperatures had resulted in no hemolysis whereas lower temperatures could have produced hemolysis It had already been established that the hemolysin in the serum of various patients was activated at different temperatures Accordingly there was a distinct possibility that the effect of carbon dioxide on the hemolytic system in the sera of various cases might appear at different temperatures However further observations suggested that a simple difference in temperature threshold was probably not the explanation for such discrepancies in the literature For example bubbling in carbon dioxide under oil at a temperature of 11 C caused no more hemolysis of cells suspended in the serum of Case 2 than allowing such suspensions to simply stand at the same temperature for the same length of time Furthermore cyanide and sulfanilamide when employed as reported before [19] did not inhibit the hemolytic activity of the serum from Case 2 These results were in striking contrast to those observed in the study of Case 1 [19] Accordingly the effect of sulfanilamide and cyanide on the Donath Landsteiner reaction was studied in more detail by using the serum from Case 1 as indicated in the following series of observations

A Experiments on the phase of action of sodium sulfanilamide and cyanide on the Donath-Landsteiner reaction

Two-tenths of a cubic centimeter of a 5 per cent suspension of normal washed Group O red blood cells were placed in each of 6 tubes (see table 4) To tube 1 was added 0 1 cc of normal saline to tubes 2 3 5 and 6 were added 0 2 cc of complement as guinea pig serum diluted 1 to 5 with saline (As complement was already present in the serum of Case 1 [see table 1] the addition of more complement was actually unnecessary) One tenth of a cubic centimeter of 0 08 M solutions of sodium cyanide and sodium sulfanilamide were added to tubes 2 and 3 respectively Five tenths of a cubic centimeter of serum from Case 1 were placed in all tubes All tubes were then chilled at 2 C for fifteen minutes At the end of that time 0 1 cc of normal saline 0 08 M sodium cyanide and 0 08 M sodium sulfanilamide were added to tubes 4 5 and 6 respectively All tubes were then incubated at 37 C for one hour

Results and conclusions Hemolysis occurred in all tubes except those chilled in the presence of sulfanilamide and cyanide Adding sulfanilamide and cyanide after

the chilling phase did not prevent *hemolysis Apparently therefore* cyanide and sulfanilamide had to be present during the chilling phase of the Donath Landsteiner reaction in order to prevent hemolysis

These observations raised the question of whether these substances prevented sensitization of red cells by the hemolysin or prevented the hemolysis of already sensitized cells The following experiment was performed in the attempt to answer this question

B *Experiment on the effect of sodium sulfanilamide and cyanide on the union of hemolysin with red blood cells*

Four tenths of a cubic centimeter of a 5 per cent suspension of normal washed Group O cells were placed in each of 3 tubes (see table 5) Four tenths of a cubic centimeter of complement in the form of guin a pig serum diluted with 1 to 5 with saline and 1 0 cc of serum from Case 1 were added Two tenths of a cubic centimeter of normal saline 0 08 M sodium cyanide and 0 08 M sodium sulfanilamide were added to tubes 1 2 and 3 respectively The tubes were then chilled at 2 C for twenty minutes centri

TABLE 4 —*Observations on the Time of Action of Inhibitors on the Donath Landsteiner Reaction*

Test tube	Case I serum	Control R B C	Complement	Normal saline	Sodium cyanide	Sodium sulfanilamide	Hemolysis
	cc	*cc*	*cc*	*cc*	*cc*	*cc*	*cc*
1	0 5	0 2		0 1			+++
2	0 5	0 2	0 2		0 1		0
3	0 5	0 2	0 2			0 1	0
4	0 5	0 2		0 1			+++
5	0 5	0 2	0 2		0 1		+++
6	0 5	0 2	0 2			0 1	+++

Procedure Test tubes 1 and 4 are controls 0 1 cc of saline sodium cyanide sodium sulfanilamide add d to test tubes 1 2 and 3 before chilling at 2 C for 15 minutes Same reagents added to test *tubes 4 5 and 6 after chilling at 2 C for 15 minutes All tubes then incubated at 37 C for one hour*

fuged in the cold by packing the tubes in ice and the supernatant pip tted off The cell were then washed in cold phosphate buffer (see table 5) The cells of tubes 2 and 3 were washed in cold phosphate buffer containing 0 008 M concentration of sodium cyanide and sodium sulfanilamide respectively i e in a known effective concentration of inhibitors to prevent any union of hemolysin with the red cells during the washing in the chilled state that might occur as the concentration of inhibitors decreased The *phosphate buffer at pH 7 2 was used to prevent the alkalinizing* effect of sodium cyanide and sodium sulfanilamide in unbuffered solution Such a high pH might inactivate complement or elute the hemolysin from the red cell After washing twice in the above solutions the cells were resuspended in normal saline and the cell suspensions were then used as shown in table 6 The cell susp nsions from each tube represented in table 5 were warmed to 27 C and divided into aliquots of 0 2 cc each To tubes 1 3 and 5 were added 0 2 cc of normal saline and to tube 2 4 and 6 were added 0 2 cc of complement in the form of guinea pig serum diluted 1 to 5 with saline The tubes ere then incubated at 37 C for one hour

Results and conclusions Hemolysis occurred as shown in table 6 This indicated that the effect of sulfanilamide and cyanide on the cells and serum was a reversible one and also that the presence of sulfanilamide and cyanide in the cold did not prevent the union of the hemolysin with the red cell The red cells had apparently

become sensitized by the hemolysin in the cold in spite of the presence of the inhibitors The subsequent withdrawal of the inhibitors allowed the hemolysis to proceed during the incubation at 37 C

These conclusions were substantiated by the following experiment

C *Experiment on the effect of sodium cyanide on the union of hemolysin with red blood cells*

TABLE 5 —*Observations on Effect of Inhibitors on Union of Hemolysin and Complement with R B C (Part A)*

Test tube	Case 1 serum	Control R B C	Compl	Normal saline	Sodium cyanide (.08 M)	Sodium sulfanilamide (.08 M)
		cc	cc	cc	cc	
1	1.0	0.4	0.4	0.2		
2	1.0	0.4	0.4		0.2	
3	1.0	0.4	0.4			0.2

Procedure Tubes chilled at 2 C for 20 mins centrifuged in cold and supernatant pipetted off
Test tube 1—R B C washed twice with 5 cc isotonic phosphate buffer (pH 7.2)
Test tube 2—R B C washed twice with 5 cc isotonic phosphate buffer (pH 7.2) containing .008 M sodium cyanide
Test tube 3—R B C washed twice with 5 cc isotonic phosphate buffer (pH 7.2) containing .008 M sodium sulfanilamide
R B C of each tube then resuspended in 0.4 cc norm saline and employed as shown in next table (part B)

TABLE 6 —*Observations on Effect of Inhibitors on Union of Hemolysin and Complement with R B C (Part B)*

Test tube	R B C resuspended in saline as prepared in part (A)			Normal saline	Compl	Hemolysis
	From T T 1	From T T 2	From T T 3			
	cc				c	
1	0.2			0.2		0
2	0.2				0.2	+++
3		0.2		0.2		0
4		0.2			0.2	++
5			0.2	0.2		0
6			0.2		0.2	++

Procedure Compl and normal saline added to test tubes at room temp (27 C) All tubes then incubated at 37 C for one hour

To 1 cc of packed Group O cells in three separate test tubes were added 0.5 cc of a buffered 0.08 M sodium cyanide solution (tubes 1 2 and 4) To another tube (tube 3) of 1 cc of packed red cells 0.5 cc of saline was added To each tube were then added 0.2 cc of guinea pig serum and 3.3 cc of the serum of Case 1 One tube containing cyanide (tube 1) was allowed to stand at 34 C The other three tubes were chilled at 3 C for thirty minutes Two of the chilled tubes (tube 2 containing cyanide and tube 3 not containing cyanide) were then centrifuged in chilled containers with packed ice The contents of the tube (tube 1 containing cyanide) not chilled were centrifuged at room temperature The supernatant in the centrifuged tubes were removed and the cells resuspended in 2.0 cc of saline The fourth tube (tube 4) contained cyanide and as a control was incubated at 37 C for one hour following chilling The supernatant from the tubes 1 2 and 3 were then dialyzed in cellophane bag against normal saline over night

at icebox temperature The dialyzed supernatants were then used in Donath Landsteiner reactions (see table 7) and titered for complement Small aliquots of the cell suspensions were incubated for one hour at 37 C in equal aliquots of Coomb s serum and with complement (see table 8)

Results and conclusions Cells chilled in the serum of Case 1 both in the presence and absence of cyanide were agglutinated in the Coomb s test and showed hemol

TABLE 7 —*Observations Shouing Cyanide did not Prevent the Union of Hemolysin with the Red Cell during Chilling*

Test t be	D alysed supernatant serum from			Complement	Saline	R B C (5 per cent suspensio)	Hem lysi
	Tube 1	Tube 2	Tube 3				
	c	*cc*	*cc*	*cc*	*cc*	*cc*	
1	0 5			0 2	0 1	0 2	4+
-		0 5		0 2	0 1	0 2	0
3			0 5	0 2	0 1	0 2	1+

Tube 1 = serum previously incubated at 34 C for 30 minutes with cyanide and red cells (see text) dialyzed and employed as shown

Tube 2 = serum previously chilled at 3 C for 30 minutes with cyanide and red cells (see text) dialyzed and employed as shown

Tube 3 = serum previously chilled at 3 C for 30 minutes with red cells but in the absence of cyanide (see text) dialyzed and employed as shown

Hemolysis as determined after chilling the suspension at 2 C for 10 minutes and incubating at 37 C for 1 hour

TABLE 8 —*Observations Shouing the Adsorption of Hemolysin by Red Cells during Chilling in both the Presence and Absence of Cyanide*

Te t t be	Wa hed ll from			C omb s se m	Compl me t	A lut at on	H molys s
	Tube 1	Tube 2	Tube 3				
1	0 1			0 1		0	
2	0 2				0 2		Neg
3		0 1		0 1		2+	
4		0 2			0 2		Pos
5			0 1	0 1		2+	
6			0 2		0 2		Pos

Tube 1 = cells previously incubated at 34 C for 30 minutes with cyanide and hemolysin washed in saline and employed as shown

Tube 2 = cells previously chilled at 3 C for 30 minutes with cyanide and hemolysin washed in saline and employed as shown

Tube 3 = cells previously chilled at 3 C for 30 minutes with hemolysin but without cyanide washed in saline and employed as shown

* Agglutination and hemolysis as determined after 1 hour incubation at 37 C

ysis on incubation with complement at 37 C after being washed in a large amount of saline (table 8) Employing the dialyzed supernatant from the chilled tubes in the Donath Landsteiner reaction showed that the hemolysin was absent or present in only a trace That the hemolysin and complement had been adsorbed and not

simply destroyed by cyanide was shown by the marked hemolytic activity in a Donath Landsteiner reaction of the dialyzed supernatant from the tubes allowed to stand at 34 C for thirty minutes Complement was present in that supernatant in a titer of 16 units per cc That the original cyanide concentration was an effective one was shown by the slight trace of hemolysis in an identical suspension (tube 4) chilled for the same period of time and incubated at 37 C for one hour Thus by a second technic adsorption of hemolysin and complement by the red cell in the presence of cyanide was demonstrated

Discussion

Both hemolysin and some component of complement were adsorbed by red blood cells from the sera of each of 2 cases in the cold phase of the Donath Landsteiner reaction This observation supports some of those reported by Cooke [20] Hoover and Stone [8] and by Dennie and Robertson [9] Cooke [20] has pointed out that although Donath and Landsteiner [1] maintained the view that cold was necessary only for the union of red blood cell and hemolysin and that complement united in the warm phase their experiments did not actually prove this Moss concluded that complement did not enter into the reaction of the cold phase (at least not permanently) His evidence consisted of the demonstration of complement in the supernatant serum after chilling with cells This does not rule out the possibility that some complement was actually used Hoover and Stone[8] reported that after chilling red cells in heat inactivated serum from a patient with paroxysmal cold hemoglobinuria incubating then in normal serum at 37 C did not cause hemolysis Washing cells in saline and rechilling and reincubating them in normal saline did cause hemolysis They interpreted their results as showing a complement factor must also be adsorbed in the cold by cells before they are susceptible to hemolysis on subsequent incubation Hemolysin and complement in the serum of Case 2 were separable by heat After inactivating the serum from Case 2 for complement the hemolysin remained Chilling and incubating cells in such inactivated serum did not cause hemolysis Nor did restoring complement to the previously inactivated serum cell suspension after chilling cause hemolysis However rechilling and reincubating at 37 C such a suspension following the addition of complement did cause hemolysis indicating complement had to be present during the cold phase of the Donath Landsteiner reaction for the hemolysin to be effective Therefore the assumption that complement acts only in the warm phase of the Donath Landsteiner reaction under these defined in vitro conditions is incorrect in at least one instance

Dennie and Robertson[9] observed that complement was necessary not only in the cold but also for the warm phase of the Donath Landsteiner reaction Although Cooke[20] concluded from his work that complement united solely in the cold some of his observations show that complement was a requisite for the warm phase also Throughout several of his experiments cells were chilled in the patient s serum and then resuspended in saline Cooke reported no hemolysis in such saline suspensions on rewarming However if both complement and hemolysin were active only in the cold then rewarming such saline suspensions should result in hemolysis Chilling cells in the presence of complement and hemolysin and resuspending them

at icebox temperature The dialyzed supernatants were then used in Donath Landsteiner reactions (see table 7) and titered for complement Small aliquots of the cell suspensions were incubated for one hour at 37 C in equal aliquots of Coomb s serum and with complement (see table 8)

Results and conclusions Cells chilled in the serum of Case 1 both in the presence and absence of cyanide were agglutinated in the Coomb s test and showed hemol

TABLE —*Observations Showing Cyanide did not Prevent the Union of Hemolysin with the Red Cell during Chilling*

Test tube	Dialysed supernatant serum from			Complement	Saline	R B C (5 per cent suspension)	Hemolysis
	Tube 1	Tube 2	Tube 3				
	cc	cc	cc	cc	cc	cc	
1	0 5			0 2	0 1	0 2	4+
2		0 5		0 2	0 1	0 2	0
3			0 5	0 2	0 1	0 2	1+

Tube 1 = serum previously incubated at 34 C for 30 minutes with cyanide and red cells (see text) dialyzed and employed as shown

Tube 2 = serum previously chilled at 3 C for 30 minutes with cyanide and red cells (see text), dialyzed and employed as shown

Tube 3 = serum previously chilled at 3 C for 30 minutes with red cells but in the absence of cyanide (see text) dialyzed and employed as shown

Hemolysis as determined after chilling the suspension at 2 C for 10 minutes and incubating at 37 C for 1 hour

TABLE 8 —*Observations Showing the Adsorption of Hemolysin by Red Cells during Chilling in both the Presence and Absence of Cyanide*

Test tube	Washed cells from			Coomb s serum	Complement	Agglutination	Hemolysis
	Tube 1	Tube 2	Tube 3				
		c	c	cc	c		
1	0 1			0 1		0	Neg
2	0 2				0 2		
3		0 1		0 1		2+	Pos.
4		0 2			0 2		
5			0 1	0 1		2+	Pos
6			0 2		0 2		

Tube 1 = cells previously incubated at 34 C for 30 minutes with cyanide and hemolysin washed in saline and employed as shown

Tube 2 = cells previously chilled at 3 C for 30 minutes with cyanide and hemolysin washed in saline and employed as shown

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Discussion

Both hemolysin and some component of complement were adsorbed by red blood cells from the sera of each of 2 cases in the cold phase of the Donath Landsteiner reaction This observation supports some of those reported by Cooke [10] Hoover and Stone [8] and by Dennie and Robertson [9] Cooke [10] has pointed out that although Donath and Landsteiner [1] maintained the view that cold was necessary only for the union of red blood cell and hemolysin and that complement united in the warm phase their experiments did not actually prove this Moss concluded that complement did not enter into the reaction of the cold phase (at least not permanently) His evidence consisted of the demonstration of complement in the supernatant serum after chilling with cells This does not rule out the possibility that some complement was actually used Hoover and Stone[8] reported that after chilling red cells in heat inactivated serum from a patient with paroxysmal cold hemoglobinuria incubating then in normal serum at 37 C did not cause hemolysis Washing cells in saline and rechilling and reincubating them in normal saline did cause hemolysis They interpreted their results as showing a complement factor must also be adsorbed in the cold by cells before they are susceptible to hemolysis on subsequent incubation Hemolysin and complement in the serum of Case 2 were separable by heat After inactivating the serum from Case 2 for complement the hemolysin remained Chilling and incubating cells in such inactivated serum did not cause hemolysis Nor did restoring complement to the previously inactivated serum-cell suspension after chilling cause hemolysis However rechilling and reincubating at 37 C such a suspension following the addition of complement did cause hemolysis indicating complement had to be present during the cold phase of the Donath Landsteiner reaction for the hemolysin to be effective Therefore the assumption that complement acts only in the warm phase of the Donath Landsteiner reaction under these defined in vitro conditions is incorrect in at least one instance

Dennie and Robertson[9] observed that complement was necessary not only in the cold but also for the warm phase of the Donath Landsteiner reaction Although Cooke [10] concluded from his work that complement united solely in the cold some of his observations show that complement was a requisite for the warm phase also Throughout several of his experiments cells were chilled in the patient s serum and then resuspended in saline Cooke reported no hemolysis in such saline suspensions on rewarming However if both complement and hemolysin were active only in the cold then rewarming such saline suspensions should result in hemolysis Chilling cells in the presence of complement and hemolysin and resuspending them

in either saline or heat inactivated complement was followed by no hemolysis on incubation at 37 C Adding intact complement to such warm suspensions however did cause hemolysis Apparently complement was necessary for the warm as well as the cold phase of the Donath Landsteiner reaction

The hemolysin in the serum of Case 1 was inactivated by heating before complement was destroyed Heating the serum of Case 2 long enough to inactivate the complement did not inactivate the hemolysin Mackenzie has also observed differences in the thermostability of hemolysins from various patients with this disease entity, and Yorke and Macfie[10] found the thermolability of the hemolysin in the serum of 1 patient actually varied from time to time

Several observers have shown that carbon dioxide may be an in vitro activating factor in this hemolytic system [3, 4] As reported previously when cells were suspended in the serum of Case 1 at 27 C with carbon dioxide in a concentration of 3 6 mEq per liter (pH 6 4) lysis rapidly occurred [19] The same procedure at 37 C did not cause hemolysis and lysis did not occur even at 27 C if the serum hemolysin had been previously removed Furthermore sulfanilamide and cyanide prevented the lysis of a Donath Landsteiner reaction when this same serum was employed As the study of other patients sera had revealed no such effect of carbon dioxide it was thought that temperature might have been a critical variable Thus workers employing lower temperatures might activate the system with carbon dioxide while those using higher in vitro temperatures might obtain negative results The upper limit of temperature at which the serum hemolysin becomes effective seems to vary from patient to patient As the carbon dioxide in vitro effect is dependent on the presence of hemolysin [19] it seemed logical that the carbon dioxide activity at a given temperature might depend on the thermal amplitude of that particular hemolysin Although such temperature factors may be important study of Case 2 suggests that they are not the only ones At a temperature (11 C) which alone activated the system in vitro the use of carbon dioxide did not increase the amount of hemolysis of cells suspended in the serum of Case 2 Mackenzie had previously reported that carbon dioxide did not activate the in vitro hemolytic system of sera from several patients with this disease whom he had studied In contrast to the effectiveness of sulfanilamide and cyanide in preventing hemolysis of cells suspended in the serum of Case 1 these inhibitors were ineffective in the prevention of hemolysis of cells suspended in the serum of Case 2 Thus the effectiveness and ineffectiveness of carbon dioxide sulfanilamide and cyanide ran parallel in the hemolytic system

Sulfanilamide and cyanide in the serum of Case 1 did not prevent the union of the hemolysin and complement with the red cell but did prevent the subsequent lysis The adsorption of the requisite serum factors for lysis by the erythrocytes when chilled in both the presence and absence of such inhibitors was shown in two ways Hemolysin and complement either decreased markedly in concentration or actually disappeared from the supernatant of all such chilled suspensions Furthermore the cells from such chilled suspensions when washed in cold saline and then incubated at 37 C with fresh complement were lysed This showed the hemolysin had acted on the cell membrane irrespective of the presence of the inhibitors Lastly if cells

chilled in the presence of cyanide and hemolysin were washed in saline and then incubated in Coomb s serum they were agglutinated This was further evidence that some serum factor had been adsorbed on the red cell Such agglutination of the cells by an anti human serum rabbit serum occurred even though the thermolabile components of complement were furnished by guinea pig serum

The prevention of lysis was not due to an inactivation of the complement necessary during the warm phase of the Donath Landsteiner reaction This was established by the lack of effect of the inhibitors when added to the red cell serum suspension after chilling but before warming The inhibitors had to be present during the entire Donath Landsteiner reaction in order to be effective This suggests the possibility that these substances may be acting at the red cell membrane and though not preventing the union of the hemolysin complement complex with the red cell may at least in some cases prevent some subsequent alteration in the spatial configuration of the hemolysin-complement red cell membrane complex that is a requisite for actual hemolysis The differences in the effectiveness of these inhibitors might therefore be associated with differences in the size of the antibody involved in various sera

Apparently the union in the cold of hemolysin and complement with the red cell membrane does not necessarily lead to hemolysis Some other step must occur That the latter may be delayed and yet be effective is indicated by the reversibility of the inhibiting effect of cyanide and sulfanilamide That the inhibiting activity of these two substances does not depend on preventing either the union of the requisite serum factors in the cold or the activity of some additional factor during the warm phase has been illustrated in three ways (1) Both hemolysin and complement disappear or at least diminish in concentration from the supernatant of a chilled suspension containing either of the two inhibitors (2) This diminution in titer is due to adsorption and not destruction as illustrated by subsequent positive Coomb s tests as well as lysis of the cells when washed and incubated in complement at 37 C (3) Finally the inhibitors do not prevent the lysis unless present *before* chilling Their ineffectiveness when added *only* during the warming phase indicates their action is not due to some effect on a serum factor necessary during that phase of the Donath Landsteiner test

Thus by elimination of several possible interpretations the question arises whether or not by acting at the cell membrane these substances simply prevent reorientation of the antibody complement complex on the cell membrane By this concept the complex could unite with the red blood cell in the presence of the inhibitors and when the latter are removed reorientation on the membrane could occur and lysis result In such a concept two possibilities would be suggested (1) carbonic anhydrase may be contained in the red blood cell membrane and (2) the differences in the effectiveness of the inhibitors from case to case may depend on the difference in the size of the hemolysin molecule in the two sera Thus the more thermolabile and possibly larger hemolysin could not reorient itself on the cell membrane in the presence of the inhibitors but the thermostabile and possible smaller antibody could As a result the inhibitors would be effective in the first instance and not in the second

Conclusions

Observations made on 2 cases of paroxysmal (cold) hemoglobinuria showed

1 Hemolysin and complement were adsorbed by erythrocytes in the cold

2 A thermolabile fraction of complement was necessary for both the cold phase and the warm phase of the Donath Landsteiner reaction in the serum of 1 case

3 Under specified in vitro conditions carbon dioxide affected the hemolytic system of 1 case and not that of the other this carbon dioxide effect was itself subject to the influence of temperature

4 Sulfanilamide and cyanide inhibited the Donath Landsteiner reaction in the serum of 1 case and not in the serum of the other

5 These inhibitors did not prevent the union in the cold of the hemolysin complement complex but did prevent the usual effect of such a union in 1 case

6 If not present during the chilling phase sulfanilamide and cyanide did not prevent hemolysis

7 Under specified conditions sulfanilamide and cyanide did not prevent the effect of the thermolabile component of complement in the warm phase of the Donath Landsteiner reaction

8 The differences in the observations of the 2 cases reported here and some apparent discrepanices cited from the literature suggest the possibility of important fundamental variants in the mechanism of the disease

Appendix

Case Histories

CASE 1

W B (J H H history numb r 359265) a 33 year old Negro was admitted with the chief complaint of upset stomach and dark urine after exposure to cold The family history was noncontributory The past history reveal d that fift n years prior to admission th patient had had a p nile lesion and was given antilu tic treatm nt for on and on half years The patient dated the pres nt illness as b ginning three years prior to admission At that tim he noted attacks of abdominal cramps nausea and vomiting following exposure to cold Thes episod s were associated with the passage of very dark urine in notic ably small quantities The attacks lasted for veral hours but the patient thought he could shorten th ir duration by drinking hot coffee or warming himself The physical examination was essentially negative The pertinent laboratory data were as follows The s rologic test for syphilis was positive There was a mild normocytic anemia The icterus index was 12 The urine as dark brown There was a 3 plus albuminuria as well as numerous red blood cells and white blood cells p r high power field The serum nonprotein nitrogen was 71 mg per cent The phenosulfonphthalein excretion was 38 per cent at the end of two hours Urea clearance was 48 per cent of normal maximum The Donath Landsteiner reaction was positive Cold agglutinins were present in a titer of 1 to 160 and were more thermostabile than the cold hemolysin The clinical diagnosis was syphilis associated with paroxysmal (cold) hemoglobinuria and renal impairment

CASE 2

M E (J H H history number 414118) a 30 year old Negress was admitted with the chief complaint of dark urine following exposure to cold The family and past histories were noncontributory The present illness apparently began t vo years prior to admission with periodic attacks of swelling of the eyelid lips periorbital tissues and fingers on exposure to cold This swelling was associated with itching of the affected parts The swelling and itching would disappear usually within t enty minutes when the patient became warm Such exposure to cold would occasionally be associated with mild transient ab-

dominal cramps There was no nausea or vomiting One year prior to admission the patient noticed that her urine became red dark and almost black after chilling The urine would remain dark for only four to six hours The patient stated that her eyes had been yellow occasionally during the past winter Drinking ice water would result in a tight sensation in her throat No dark urine had been noted following consumption of cold drink or food The physical examination was essentially negative except for slight pallor of the nail beds and mucous membranes The significant laboratory data were as follows The serologic test for syphilis was positive There was a moderate macrocytic anemia The icterus index was 15 Cold agglutinins were present in a titer of 1 to 320 The urine was dark brown and guaiac positive There were occasional red blood cells and white blood cells per high power field The Donath Landsteiner test was positive The clinical diagnosis was syphilis associated with paroxysmal (cold) hemoglobinuria and angioneurotic edema following exposure to cold

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PART III

ANEMIA
OTHER THAN PERNICIOUS ANEMIA
AND HEMOLYTIC ANEMIA

23

CLINICAL SIGNIFICANCE OF CARDIAC AND RESPIRATORY ADJUSTMENTS IN CHRONIC ANEMIA

By Herrman L. Blumgart M D and Mark D Altschule M D

I Introduction

THE SUPPLY of oxygen to the tissues depends on the oxygen carrying capacity of the blood and the ability of the cardio respiratory system to aerate and to transport the blood to each living cell of the body The purpose of this communication is to review the status of the cardio-respiratory system and the related manifold compensatory mechanisms which provide a maximal supply of oxygen to the tissues in the presence of anemia An understanding of these adjustments is necessarily based on knowledge of the changes in cardio respiratory dynamics and on an appreciation of the nature of the clinical manifestations

Most of the physiologic studies reviewed here have been published during the past twenty years These two decades were most fruitful for it was during this period that it became possible to study the same patients before and after effective therapy This was the direct consequence of the discovery by Minot of the therapeutic effectiveness of liver and the studies in iron therapy which were also carried out to an important extent in his laboratories

II Changes in Cardiovascular Dynamics

The Minute Volume Output of the Heart

Numerous investigations have shown that with anemia there is an increased cardiac minute volume output of the heart The relation between the severity of anemia and the degree of increase of the minute volume output observed in different studies has not however been uniform Similarly the relation of the changes in minute volume output to other aspects of circulatory dynamics has also varied in different investigations This is hardly surprising when one considers the number of variable factors involved

The method used to measure the minute volume output of the heart in man have been of necessity indirect and have depended until recently on respiratory techniques The complexity and technical variations of these methods have made quantitative comparison difficult and the number of measurements inevitably meager for statistical study In many communications the actual minute volume output values have been related directly to the hemoglobin concentration of the blood without regard for the fact that variations in cardiac minute volume output in normal people tend to be proportional to variations of the surface area and of the oxygen consumption Anemias moreover are frequently related to and indeed may be caused by diseases which in themselves affect cardiac output because of fever or nutritional disturbances

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Plesch[86] in 1909 observed that the cardiac output increased when there was a decreased hemoglobin concentration of the blood Utilizing the Fick principle he found that the increase in cardiac output roughly paralleled the decrease in hemoglobin Confirmatory results have been reported by other investigators[15, 37, 38, 40, 63, 75, 80, 9, 97, 100, 103] who used a variety of methods but Kininmonth s results[6] are discordant Several excellent studies in which measurements were made in the same subjects both in the anemic state and after improvement are of particular interest [40, 80, 92, 101, 103] On the basis of twenty two observations in 8 individuals Richards and Strauss[9] concluded that there was a definite though not entirely constant tendency for the cardiac output to increase with decreasing hemoglobin concentrations this general relationship as well as the variation disclosed in the individual instances applied to both the cardiac minute volume output and to the cardiac index or the ratio between cardiac output and surface area Results obtained by more accurate methods based on cardiac catheterization have become available recently In a series of anemic patients studied by Sharpey Schafer [97] cardiac output was elevated 50 to 150 per cent over the normal average of 5 3 liters per minute and generally was greatest at the lowest levels of hemoglobin concentration Similar results have been reported by others utilizing the same technic [15]

There has been no uniform agreement as to the level to which the hemoglobin concentration falls before the cardiac output is definitely increased Dautrebande[2] observed an increase only when the hemoglobin fell below 50 per cent Although others have expressed the opinion that a considerably lesser degree of anemia may cause a measurable rise in cardiac output [75] no unequivocal rise in the cardiac output was observed in the studies by Brannon and his associates[15] until the hemoglobin concentration was less than seven grams per cent

Relation Between Cardiac Minute Volume Output Metabolic Rate and Coefficient of Oxygen Utilization

When the oxygen carrying capacity of the blood is diminished in a patient with anemia two mechanisms are available to maintain an adequate supply of oxygen to the tissues these mechanisms may act singly or together The first mechanism which may compensate for deficient concentration of hemoglobin consists of an increased delivery of blood to the tissues consequent to increased cardiac output If the concentration of hemoglobin were 50 per cent of normal and the cardiac minute volume output and blood flow were doubled the amount of oxygen withdrawn from each cubic centimeter of blood in the capillaries might be one half the normal but the total amount of oxygen given off to the tissues would be unchanged Despite the low arterial blood oxygen content the venous blood oxygen concentration would remain normal Since the venous blood oxygen tension reflects that of the tissues it is clear that tissue anoxia would be prevented Sole reliance on this mechanism would require the heart to expend a greatly excessive amount of

energy in order to prevent tissue anoxia and the circulatory reserve would be en croached upon to a marked degree

The minute volume output of the heart other factors being equal is also related to the total oxygen consumption of the body If the total oxygen consumption of the body were markedly increased in the presence of anemia part of the increased minute volume output of the heart would be attributable to this factor Most ob servers however have found normal values while others have observed small in creases in some instances this will be discussed below Thus the increased output observed in anemia can be ascribed only slightly if at all to increased total oxygen consumption of the body

The second mechanism available for maintainance of an adequate supply of oxy gen to the tissues consists of more nearly complete abstraction of oxygen from the blood as it passes through the capillaries Normally 100 cc of arterial blood con tains approximately 21 cc of oxygen Under normal basal conditions only about 5 5 cc i e approximately 30 per cent are removed from the blood as it passes through the capillaries The remaining 15 5 cc may be regarded as reserve oxygen which can be called upon during exercise or other unusual states to prevent as phyxia of the tissues The anemic patient to the extent to which he relies on the mechanism of more complete oxygen abstraction diminishes his reserve oxygen and sacrifices this factor of safety the degree of sacrifice would depend upon the severity of the anemia Moreover the gradient between the oxygen tensions of capillary blood and that of the cells of the tissues must be less under these circum stances

The data available in the literature clearly demonstrate that the burden of ane mia on the cardiovascular system is distributed part being assumed by an increase in the cardiac output per minute and a part by the increased percentage i e co efficient of utilization of oxygen by the tissues The actual amount of oxygen ab stracted from the blood as it courses through the capillaries of an anemic patient is less than normal but the ratio between the amount abstracted and the subnormal amount initially present in the arterial blood is actually greater and is reflected in the increased percentage of oxygen utilization uniformly observed in patients with severe anemia It should be noted that normally an A V oxygen difference of 5 5 grams per 100 cc of blood signifies an A V difference of approximately 30 per cent of the available oxygen If there were no increase in cardiac output in severe ane mias with less than 30 per cent hemoglobin the amount of oxygen which could be delivered to the tissues even with 100 per cent abstraction of oxygen would be less than the normal oxygen supply In the most severe degrees of anemias 80 to 90 per cent of available oxygen is removed were it not for the increased percentile ab straction higher values of cardiac output than those actually observed would be required to maintain adequate minute oxygen supply Thus increased cardiac out put and increased percentile removal of available oxygen are two adjustments which serve to maintain adequate oxygen supply

The above considerations bear on the interesting question of what should be con sidered the normal cardiac output a concept which has important theoretic as

well as practical clinical implications The normal cardiac output must be considered the cardiac output which is found in healthy subjects at rest and free of discomfort or emotional tension at medium ambient temperatures and with normal rates of body oxygen consumption The normal cardiac output varies considerably but is proportional to the surface area the cardiac index i e ratio of cardiac output per minute to surface area being relatively constant in all normal subjects The cardiac index is proportional to metabolic rate In the presence of congestive failure due solely to cardiac disease there is a decreased cardiac index in relation to bodily requirements for oxygen which remain unchanged or are even increased [2] When however anemia supervenes in the presence of congestive failure the index is not as low as one would anticipate on the basis of congestive failure alone the cardiac output being somewhat increased rather than diminished in relation to total body oxygen consumption of normal subjects For example in the presence of severe anemia with arterial oxygen content of five volumes per cent the cardiac index is approximately seven [16] This elevation of the cardiac index to more than twice the normal of healthy subjects is the increase whereby the requirements of the body for blood and oxygen are met An elevation less than this constitutes circulatory insufficiency even though the output at such times may be larger than that of normal subjects When the cardiac index is below that indicated for the degree of anemia even though markedly above the so called normal of healthy individuals the circulation may be insufficient in relation to the increased demands of anemia congestive failure may ensue

In the presence of lowered oxygen consumption in anemic patients the expected increased cardiac output may not always be present Thus Starr et al [100] state The two cases of anemia did not show the increased cardiac output which we expected Both of them appear to have reduced their basal metabolism to a point where a normal cardiac output will carry the necessary oxygen In one of these patients starvation may well have been the cause of this decrease This method of compensating for anemia is not that usually described Similarly in patients with anemia of myxedema the cardiac output may be low but adequate for the lowered metabolic requirements congestive failure does not develop

This general principle applies equally to other conditions which demand an increased output such as thyrotoxicosis arterio venous aneurisms pregnancy febrile states and indeed exercise

The Velocity of Blood Flow

The relation between volume flow and velocity flow of liquids of fixed viscosity through tubes of known diameter is a simple one and is expressed by the equation $V = A/\pi r$ where V is velocity flow expressed in seconds A is the volume flow per second and r is the radius of the tube If other factors remain equal an increase in volume flow will be accompanied by a proportional increase in the velocity of blood flow With the somewhat decreased viscosity of blood in anemia an additional factor tending to increase the velocity of blood flow is operative The extent to which pulmonary blood flow is accelerated in the presence of anemia was studied by

Blumgart Gargill and Gilligan[11] using the radioactive method Thirty two complete series of measurements were made in 29 subjects with pernicious anemia and with anemia secondary to a variety of diseases The results showed that while there were considerable variations the velocity of blood flow through the lungs in these patients generally tended to increase in proportion to the degree of anemia A linear relationship between the increased cardiac output and accelerated velocity of blood flow was observed by Stewart et al [103] the greater the cardiac output the shorter the circulation time The increase in velocity observed by the latter investigators was of somewhat greater magnitude than that reported by Tarr Oppenheimer and Sager [106] but similar to those results recorded by Blumgart et al With increased speed of blood flow through the lungs accelerated pulse rates were observed Other studies of circulation time in patients with anemia have been reported by many authors[4, 9, 10, 13, 33, 36, 35, 56, 60, 63, 66, 70, 71, 4, 84, 106, 11] all are in accord with the above

Regulation of Peripheral Blood Flow

Calculation of the average peripheral resistance throughout the body in patients with anemia indicates that a definite decrease is present However the state of the small blood vessels is not uniform everywhere Thus plethysmographic measurements of blood flow in the arms[1] and studies based on estimation of local arterio-venous blood oxygen or carbon dioxide difference in the arms[3, 25, 30, 43, 51, 6, 79, 86, 9] show accelerated flow while blood flow in the hands is diminished[1, 104] in anemic patients Direct observations of the capillaries in the skin of the fingernail fold show marked vasoconstriction[30, 50] and flow is slow [30] The occurrence of increased peripheral flow everywhere except in the hands is identical with the pattern of flow seen in simple anoxia such as is produced in normal subjects breathing air deficient in oxygen

Measurements of antecubital and mixed venous blood oxygen content yield very low values [3, 15, 25, 51, 76, 79, 86] In spite of the fact that the tissues are given enough oxygen for their basal requirements as shown by the fact that total oxygen consumption is not lowered in anemia the low tissue oxygen tensions which exist result in a diminution of the margin of safety The occurrence of intermittent claudication of the calves in patients with anemia has been emphasized by Pickering and Wayne [85] In accord with the concept that the tissues are anoxic is the fact that blood lactic acid values are often elevated in patients with anemia[58] and the ability of the body to metabolize intravenously injected lactate is impaired [21, 45] After exercise the oxygen debt is greatly increased in anemic patients[81] presumably as a consequence of the accumulation of excessively large amounts of lactic acid

Visceral Blood Flow

1 *Kidneys* The observations of Bradley and Bradley [14] on renal blood flow in anemia are of interest in that they afford data indicating the presence of selective changes in vasomotor activity The renal blood flow is greatly diminished in patients with chronic anemia evidently as a consequence of localized vasocon

striction Calculations of the filtration fraction suggest that afferent vasoconstriction is somewhat more marked than that in the efferent arterioles of the glomeruli Although flow of blood through the kidneys is reduced by a third or a half the amount of *plasma* presented for filtration to the glomeruli per unit of time as a rule is almost normal because of the low hematocrit values which occur in anemia Accordingly nitrogen retention is uncommon On the other hand the observed reduction in blood flow may be related to salt retention observed by Strauss and Fox[105] in patients with anemia (see below) Evidence of impaired tubular function presumably consequent to anoxia is also presented by the Bradleys [14]

2 *Brain* Himwich and Fazekas[48] recorded observations on arterial and jugular venous blood gas concentrations in one anemic patient which indicate that the flow of blood through the brain is abnormally rapid in anemia the venous blood oxygen concentration however and presumably the tissue oxygen tension in the brain is very low These authors ascribed the development of mental symptoms in their patient to anoxia

The Metabolic Rate in Anemia

A review of the available data indicates there are no striking deviations from the normal total oxygen consumption of the body as a consequence of anemia per se The problem is complicated by the fact that the metabolism of the body may be influenced considerably by the disease which causes the anemia [26] In pernicious anemia Boothby and Sandiford[12] observed that approximately 10 per cent of their patients had a metabolic rate above plus 20 per cent Similarly an average increase in basal metabolic rate of plus 20 per cent was observed in the 5 patients with pernicious anemia studied by Stewart et al [103] a decrease to an average of plus 6 per cent occurring during remission induced by treatment In patients with iron deficiency anemia the metabolic rate is increased less frequently and is often normal or below normal In the 18 patients studied by Brannon et al [15] the average metabolic rate in patients with less than 7 grams per cent of hemoglobin was plus 13 as compared with plus 5 per cent in patients with hemoglobin between 7 and 13 grams and minus 7 per cent in their normal subjects In general the rise in oxygen consumption by the body in anemia is at most small or frequently absent even when present the increase in cardiac output and velocity of blood flow can be attributed only in small part to this rise in oxygen consumption

Blood Volume

Neither the carbon monoxide method nor the dye methods measure absolute blood volume in normal subjects experience with pathologic subjects tends to confirm and extend this conclusion Either method may on occasion give the larger value and it is not possible to assert that either one consistently measures absolute blood volume both methods apparently tend to exaggerate the true blood volume The dye method also generally gives excessive values for the plasma volume [83] It is fairly clear however that most methods give fairly reliable qualitative but not quantitative measures of the relative plasma volume Observations by various investigators of the blood volume in anemia reveal that the blood volume is some

what reduced[11, 30, 31, 35, 36, 97] the most thorough studies are those of Gibson et al [31, 35] The mean plasma volume per kilogram of body weight is usually within the limits of normal however so that the diminution is a reflection of reduction in total circulating red cell mass

III Changes in Respiration

Alterations in Ventilation

The respiratory minute volume is often increased in anemia even beyond what might be expected in the presence of occasional elevations of the metabolic rate[8, 16, 57, 64, 66] some authors have found no striking change in certain instances [3, 92] Increases in rate and in depth of respiration participate in the elevated respiratory volume Although these findings suggest the effects of simple anoxia it is clear that other influences operate in patients with anemia

All authors who have measured the vital capacity in patients with anemia have found lowered values in many of their subjects [11, 57, 64, 87] More detailed studies involving estimation of the subdivisions of the vital capacity i e the reserve and complemental air volumes show these to be lowered likewise [64, 87] The residual air is increased somewhat [64, 87] These deviations from the normal are similar to those observed in pulmonary congestion or edema and denote a loss of elasticity and expandability Changes of this type favor the occurrence of exertional dyspnea

Blood Gases and Their Relation to Dyspnea

Studies of the blood gases in anemia are of importance in understanding the dyspnea which may occur in this disorder Arterial anoxia obviously must exist when the hemoglobin level is reduced Although some of the earlier observers reported also that low arterial blood oxygen saturation was common[55, 86] most workers agree that the saturation is normal in patients at rest [43, 49, 51, 79] Accordingly it is apparent that the degree of pulmonary congestion and/or edema present in severe anemia is not sufficient to interfere with maximal oxygenation of the blood On the other hand during exertion arterial blood oxygen saturation falls markedly [49] indicating inefficiency of the respiratory mechanisms under conditions of strain The reduced oxygen saturation of the arterial blood conceivably might be due to some qualitative alteration of the hemoglobin in anemia however the oxygen dissociation curve of blood from anemic patients is normal at all pH levels found in man [5, 91, 99]

As pointed out above the tissue oxygen tensions or at least the gradient between blood and tissues must be greatly lowered This phenomenon exists in the brain as was shown by Himwich and Fazekas[48] and provides an additional mechanism for hyperventilation

Another group of factors important in consideration of the respiratory dynamics of anemia is related to peculiarities of carbon dioxide transport in that disorder Joffe and Poulton[59] showed by means of experiments in vitro hat erythrocytes are important in carrying carbon dioxide and Smith Means and Woodwell[98] further showed that red cells in vivo may actually carry most of the carbon dioxide given off by the tissues Accordingly it is clear that a fall in erythrocyte count must im

striction Calculations of the filtration fraction suggest that afferent vasoconstriction is somewhat more marked than that in the efferent arterioles of the glomeruli Although flow of blood through the kidneys is reduced by a third or a half the amount of *plasma* presented for filtration to the glomeruli per unit of time as a rule is almost normal because of the low hematocrit values which occur in anemia Accordingly nitrogen retention is uncommon On the other hand the observed reduction in blood flow may be related to salt retention observed by Strauss and Fox[105] in patients with anemia (see below) Evidence of impaired tubular function presumably consequent to anoxia is also presented by the Bradleys[14]

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On the other hand patients with Addisonian pernicious anemia may have no deficiency in blood carbonic anhydrase activity[87, 88, 72] and consequently suffer from no impairment of carbon dioxide transport and excretion from lack of the enzyme itself The reason for this difference between macrocytic and micro or normocytic anemias is not clear

In summary the following factors many of which are closely interrelated are operative in the production of dyspnea in anemic patients the increased respiratory minute volume the decreased vital capacity and its subdivisions abnormalities in carbon dioxide transport and dissociation reduced arterial oxygen capacity and the decreased blood oxygen saturation during effort the frequently observed elevated blood lactic acid values

IV Clinical Manifestations of Chronic Anemia

Cardiovascular and Respiratory Symptoms and Signs in Anemia

With the exception of active rheumatic infection bacterial endocarditis periarteritis nodosa disseminated lupus erythematosis and advanced organic tricuspid disease organic cardiovascular disease does not cause anemia Various cardiovascular symptoms and signs occur however in the presence of anemia and have attracted the interest of many observers for more than a century Practically all investigators have concerned themselves with isolated aspects of the problem Excellent comprehensive clinical studies of the cardiovascular system in anemia were however presented by Ellis and Faulkner in 1939 [8] and by Wintrobe[113] and Hunter in 1946 [51] the last named also providing an extensive bibliography which will therefore not be repeated here Wintrobe[113] also includes an excellent digest of some of the physiologic adjustments of the cardiovascular system in anemia

1 *Symptoms* Lassitude anorexia dizziness palpitation and breathlessness on exertion generally are found in patients with moderate or severe degrees of anemia In even the severer grades of anemia however breathlessness is rarely present at rest and orthopnea and paroxysmal dyspnea are absent Palpitation likewise is generally experienced only with exertion and implies an increase in the rate as well as in the force of the cardiac impulse Anginal pain may occur particularly on effort

2 *Physical signs* Pallor of the skin and mucous membranes changes in the tongue and finger nails and slight degrees of edema particularly over the sacrum and lower legs are characteristic of the anemic state Increased vigor of arterial pulsation with a widened pulse pressure was frequently observed by Sharpey Schafer[97] who also described capillary pulsation in the finger tips In the severer grades of anemia pistol shot sound over the larger arteries a positive Duroziez s sign and systolic murmurs on auscultation may be manifest The possibility that these evidences of vasodilatation may be related not to anemia but to associated fever or beriberi must be borne in mind

3 *Cardiac enlargement* Bamberger in 1857[6] and Friedreich in 1861[3] commented on the pathologic findings of fatty infiltration and degeneration together with dilatation and increased weight of the heart in patients with anemia Ball[5] was the first to demonstrate an increase in heart size by x ray measurements with return to

pair transport of that gas There is an apparent contradiction in this concept in the fact that the whole blood of anemic patients has a carbon dioxide combining power which is in or even above the upper normal range[3, 19, 3, 55, 54, 79, 93] the carbon dioxide combining power is estimated using whole blood and since each unit of the blood in anemic patients contains relatively more plasma and fewer cells than normal the great carrying capacity for carbon dioxide of plasma as compared to cells which exists normally results in the small increase observed in the whole blood However the role of plasma in the transport of carbon dioxide given off by the tissues may be minor that of the erythrocytes more important this is evidenced by the fact that plasma and red cells do not participate equally in the changes to be described [5, 47] The loss in anemia of much of the buffering action normally provided by hemoglobin in the red cells and the resultant flattening of the carbon dioxide dissociation curve[7, 19, 5, 44, 61, 78, 8, 109] is highly significant a given amount of carbon dioxide entering the blood causes much greater increases in carbon dioxide tension and hydrogen ion concentration than occurs when the blood hemoglobin concentration is normal Hyperventilation which makes the arterial blood somewhat alkalotic in patients with anemia [7, 19, 3, 25, 9] enables the blood as it traverses the tissues to accomodate this tendency toward acidosis the arterio venous difference for pH is thereby increased[3, 7, 43, 9] but the pH of the venous blood [23] and presumably of the tissues remains normal

It has been noted that some types of chronic anemia are associated with less exertional dyspnea than others of the same degree and chronicity but of different types Clearly factors other than those discussed above must be important in this regard In addition to hemoglobin the red cells contain other respiratory enzymes including carbonic anhydrase The latter catalyzes the reaction $HCO_3 \rightleftarrows CO + H O$ in either direction depending on concentrations of the substrate materials Earlier work with this enzyme tended to minimize its importance in the etiology of dyspnea because according to the methods used in the past there is such an enormous excess of this enzyme that it was considered impossible that a deficiency could ever exist in the adult at least There are however certain errors in these older methods which led to the finding of falsely high values in normal blood [7] By the use of a newer method it has been found that the amount of activity of the enzyme in normal blood is so small that a decrease because of disease would lead to serious impairment of the rate of absorption of carbon dioxide by the blood in passing through the tissues and the release of carbon dioxide from the blood in passing through the lungs [7] Several workers[6, 87, 68, 7] have studied the relation between carbonic anhydrase activity and hemoglobin content of the blood in normal subjects and patients with anemia It has been found that anemias due to blood loss malnutrition chronic infection uremia or leukemia are associated with a proportional reduction in blood carbonic anhydrase activity which is parallel to the decrease in hemoglobin level [7] Patients with any of these types of anemias therefore not only have decreased oxygen carrying capacity but also may have a deficiency in ability to take up carbon dioxide from the tissues and to release it in the lungs Therefore not only is anoxia responsible for their hyperventilation and dyspnea on exertion but carbon dioxide accumulation in the tissues is probably also a factor

times they may be rough or even rumbling and may be accompanied by diastolic murmurs Attention should be called to the fact that in rare instances a blowing diastolic murmur may also be heard along the left border of the sternum characteristic of aortic insufficiency Most observers have encountered these diastolic murmurs only rarely While Goldstein and Boas[39] reported an incidence of 10 per cent of these murmurs in 39 cases of anemia these diastolic murmurs occurred only in association with severe degrees of anemia The blowing apical systolic murmurs tend to be louder longer and less affected by posture and by respiration than the same systolic murmurs encountered in healthy young persons They are usually not transmitted to the axilla When however they have a rough rumbling quality and are associated with a booming first sound and the slight or moderate cardiac enlargement seen in the presence of moderate or severe anemia the differentiation from structural mitral disease may offer difficulty and indeed the distinction may be impossible In such unusual instances decision may have to be deferred until further observations are made when the anemia is alleviated [113]

It is of interest that of the 34 patients studied by Hunter[54] while they were moderately or severely anemic and again after treatment 30 showed cardiac murmurs always systolic in time except in one case which showed an early diastolic murmur and another a pre-systolic murmur Six murmurs were described as faint 16 as moderate and 9 as rough In 29 cases murmurs were heard at the apex in 21 at the pulmonary area and in 6 in the aortic area Twenty had murmurs at more than one site i e mitral and pulmonary in 14 mitral pulmonary and aortic in 6 An apical murmur alone was present in 9 cases and a pulmonary murmur in 1 Aortic murmurs were always accompanied by both pulmonary and mitral murmurs The louder murmurs were heard in two and often three areas and the softer in single areas generally the mitral When multiple murmurs were present they were loudest at the apex then in the pulmonary area and least loud in the aortic area The murmurs were usually diminished when the patients were in the erect position especially when the intensity had lessened after treatment In 9 patients the murmurs though not accompanied by thrills were loud enough to raise a suspicion of valvular disease although their distribution made such a diagnosis unlikely The two diastolic murmurs one late or pre systolic at the apex and the other early in the fourth left interspace near the sternum led to diagnoses of mitral stenosis and aortic incompetence respectively until their disappearance with treatment indicated their hemic origin The incidence of murmurs was not directly related to the severity of the anemia for they were often conspicuous when it was slight and absent when it was considerable The duration of the anemia seemed more important [54, 113] The patients described by Schwartz and Legere[98] illustrate in striking fashion the difficulties which sometimes arise in the differentiation of cardiovascular changes due to anemia from those consequent to serious organic heart disease

The wide spread belief that cardiac enlargement is the cause of hemic murmurs was not confirmed by Hunter [54] since their association was inconstant and noticeable murmurs were heard when the heart was normal in size and occasionally absent when there was enlargement It is probable that acceleration of blood flow is an important cause of the murmurs noted however the decreased viscosity of the

normal after recovery this finding has been confirmed by many observers [28 41 54 89 107] Twelve of the 34 patients studied by Hunter[54] showed definite cardiac enlargement during anemia, with return to normal size in all but 3 following appropriate treatment In some patients with slight or doubtful degrees of enlargement a decrease in size following disappearance of the anemia was noted The reduction in size was always generalized but in addition any straightening of the left border which had been initially present was replaced by a normal concavity Cardiac enlargement tends to occur more frequently in patients with particularly low hemoglobin levels and according to Hunter,[54] there is a decrease in enlargement when the hemoglobin percentage rises to the levels of 60 to 95 per cent variation being observed in different subjects Regression in cardiac size usually occurred early often within two or three weeks of the beginning of treatment and at hemoglobin levels still substantially below normal

Cardiac hypertrophy however is also frequently present [17 18 28 30 54 89 90] The available data seemingly support the opinion of Porter[89 90] that primary cardiac dilatation is a physiologic adjustment resulting from the increased cardiac output in the presence of a supply of anemic blood through the coronary arteries to the myocardium If the anemia is rectified early dilatation disappears and heart size returns to normal If however prolonged dilatation continues with stretching and injury to the myocardial fibers cardiac hypertrophy inevitably develops [24] The degree of cardiac hypertrophy is usually not marked but an instance is cited by Cabot[17 18] in which the heart weighed 710 grams in the absence of arterial hypertension and coronary arteriosclerosis the heart of a patient observed by Porter weighed 630 grams [89]

In general it may be said that both dilatation and consequent hypertrophy undoubtedly occur in patients with anemia but that there is no direct relation between the incidence or degree of enlargement and the severity of the anemia With the return of the hemoglobin levels toward normal cardiac size usually returns to within normal limits although occasional instances of persistent slight enlargement may be witnessed In addition to dilatation and hypertrophy the anemic heart muscle undergoes a form of fatty degeneration characterized by yellow streaking (tigering) clearly visible on the endocardial surface

4 *Heart sounds* A loud and booming first sound is frequently heard in patients with anemia but no direct relation to the hemoglobin level or the duration of the anemia is apparent [54 89 90] The prolongation and accentuation of the apical first sound may closely resemble the characteristic first sound in mitral stenosis The aortic and pulmonic sounds are usually not altered but in some patients an additional third heart sound is heard at the apex This latter finding has been corroborated by phonocardiography and must be considered abnormal since the patients were over 30 years of age [54] Both the booming first sound and the presence of the third sound were associated with a moderate tachycardia and disappeared when the pulse rate slowed

5 *Cardiac murmurs* That a blowing systolic murmur commonly is heard at the apex or over the mitral area in anemic patients is generally appreciated Occasionally however such murmurs are heard over the aortic and pulmonic areas and at

gram was sometimes observed after treatment although this did not always occur even when there was no reason to suspect other forms of heart disease In 2 of the 25 patients studied electrocardiographically by Hunter [54] gross abnormalities were apparent but in spite of successful treatment of the anemia the electrocardiogram was unchanged on re examination a year later both patients were in the fifth decade and the changes may have been consequent to coronary arteriosclerosis or other lesions

The possibility of vitamin B deficiency digitalis administration and in some patients the effect of the disease responsible for the anemia are difficult to exclude in some of the reported cases although these factors were evidently ruled out in the studies by Ellis and Faulkner [8] and Hunter [54]

The electrocardiographic changes frequently apparent in chronic anemia are similar to those observed during acute anoxia That anoxia is an important factor is further supported by the frequently transitory character of the changes in moderate or severe anemia In some patients the electrocardiographic changes may represent the summation of anoxia coexistent coronary arteriosclerosis and fatty changes in the myocardium

It is not surprising that the effects of anoxia are prone to occur in the heart during chronic anemia for even under normal conditions the abstraction of oxygen from the blood as it flows through the heart is relatively great Thus mixed venous blood in the right chambers contains approximately 15 volumes per cent of oxygen and as stated above this amount of oxygen represents a reserve factor On the other hand blood from the coronary sinus obtained in observations in man by catheterization technics contains only approximately two volumes per cent The abnormal reduction of this reserve factor in the heart the lowering of blood pressure commonly observed in anemia and the increased work of the heart attendant to the increased output predispose to anoxia

Angina Factors and Severe Anemia

Herrick[46, 47] drew attention to patients with severe anemia who developed angina pectoris and who experienced relief when the anemia improved this was confirmed subsequently by many other investigators [16, 0, 7, 8, 54, 61, 8, 85, 111, 114] It is to be expected that in any large series of patients with anemia an occasional instance of coincidental angina pectoris might be encountered The fact however that angina pectoris first appears in some patients with the development of severe anemia and is alleviated by appropriate treatment of the anemia bespeaks an etiologic interrelationship Such patients are encountered only occasionally and are almost always in the age group in which coronary arteriosclerosis is more common Cabot[17] and Elliot [7] however have both reported cases in which no evidence of coronary artery disease was found postmortem In most patients of this type however it is probable that the heart is damaged to so slight an extent that it is able to maintain an adequate blood flow provided that the oxygen carrying capacity of the blood is normal In the presence of anemia however the increased amount of work necessary to compensate for this condition cannot be accomplished readily particularly since the blood supply to the heart is affected in common with

blood which accompanies anemia favors the development of eddies and therefore of murmurs as Wiggers[110] pointed out

As mentioned above cardiac enlargement usually diminishes rapidly while the murmurs tend to persist over a longer period of time Treatment usually effects a lessening in the intensity of the murmur and most murmurs disappear or become negligible when the blood findings return to normal In a few instances however such murmurs may persist for several months before disappearing

6 *Tachycardia* Moderate increase in the cardiac rate is nearly always present with moderate or severe anemia the rate rising to 90 to 100 The relation between the rise in pulse rate and the degree of anemia is variable In studies on the velocity of blood flow through the lungs Blumgart et al [11] observed that the pulse rate was more closely related to changes in the velocity of the pulmonary circulation than to variations in the degree of anemia This result is hardly surprising since the car diac rate and the velocity of blood flow are both characteristics of the general circulatory adjustment and as such are more closely related physiologically to each other than to change in the oxygen carrying capacity of the blood

7 *Arterial blood pressure* In the presence of moderate or severe anemia a lowered blood pressure is commonly observed with a subsequent rise as recovery from the anemia occurs [15, 85, 103] Although the 5 patients studied by Stewart et al [103] ex hibited a rise in arterial pressures amounting in 3 of their patients to as much as 50 to 80 millimeters and 30 to 46 millimeters of mercury in the systolic and diastolic pressures respectively other workers found lesser changes Similar though not as striking or uniform findings were reported by Brannon et al [15] while Bradley and Bradley [14] found no consistent change This tendency for the blood pressure to rise with recovery from anemia occasionally counterbalances the decreased work of the heart which also occurs at this time due to lessened volume output and velocity of blood flow during recovery it may result in the work of the heart being approxi mately the same in some patients during anemia as after recovery [103] The rise in arterial pressure which may develop is a reflection of the increase in total peripheral resistance which occurs when the need for visceral vasodilatation is no longer pres ent as the hemoglobin level approaches normal The somewhat widened pulse pres sure sometimes seen in anemia with narrowing of the span after recovery is in accord with this concept

8 *Venous pressure* The right auricular pressure in patients studied by Sharpey Schafer[9] was usually a high normal A slight elevation was noted in one of 5 patients by Stewart et al [103] while no significant alterations were noted in the other 4 patients No elevation was found by other authors [33, 106] Sharpey Schafer[97] be lieves that the increased output is achieved mainly by the raised venous pressure a concept which requires further verification

9 *Electrocardiographic changes* Numerous studies of the electrocardiogram in chronic anemia indicate that while abnormalities occur in approximately a quarter or more of such patients they are minor in degree and are not specific for anemia Prolongation of the QT interval [107] flattening or inversion of T in one or more leads [7] low amplitude [108, 111] transitory prolongation of the P R interval [45] depres sion of the ST segment [5] have been noted A return to normal of the electrocardio

gram was sometimes observed after treatment although this did not always occur even when there was no reason to suspect other forms of heart disease In 2 of the 25 patients studied electrocardiographically by Hunter [81] gross abnormalities were apparent but in spite of successful treatment of the anemia the electrocardiogram was unchanged on re examination a year later both patients were in the fifth decade and the changes may have been consequent to coronary arteriosclerosis or other lesions

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that of the rest of the body Furthermore the normally large utilization of available oxygen by the heart predisposes to the induction of ischemic muscle pain in severe anemia

Congestive Failure in Anemia

The necessity for the maintenance of the cardiac output and cardiac work at an abnormally high level for long periods of time and the delivery to the myocardium of blood deficient in oxygen together favor the occurrence of myocardial insufficiency Other factors favoring it are present when the patient with severe anemia also is in the age group in which coronary artery disease and hypertension occur as is common in pernicious anemia and anemia due to carcinoma Nephritic anemia usually is also complicated by the presence of hypertension The coexistence of organic cardiovascular disease added strain upon the heart due to anemia and anoxia of the myocardium are especially prone to result in congestive heart failure When this syndrome develops the circulatory dynamics change [97] The right auricular and venous pressures become elevated the lungs become more congested and the cardiac output although often still elevated in comparison to the normal becomes lowered relative to the values which ordinarily obtain with a given degree of anemia The arm to tongue circulation time in such instances represents the resultant of two opposing factors i e a tendency toward slowing due to congestive failure on one hand and toward acceleration due to anemia on the other it often lies in or close to the normal range as a consequence Patients in whom myocardial insufficiency develops as a consequence of anemia have aggravation of their antecedent exertional dyspnea as is to be expected from the superimposition of the additional mechanisms favoring dyspnea which occur in chronic congestive failure Such patients are likely to respond poorly to digitalis unless the anemia is corrected On the other hand since congestive heart failure develops in patients with anemia when the heart is less seriously damaged than in those in whom cardiac decompensation is referable to heart disease alone the ultimate outlook assuming adequate therapy of the anemia is better in the former than in the latter

Dyspnea is not necessarily an indication of congestive failure Dyspnea may be consequent to anemia per se and as such may be the resultant of the action of a number of factors each of which has been discussed above These include the effect of low arterial blood oxygen concentrations on the carotid body the effect of low tissue oxygen tensions directly on the brain the effect of low oxygen tension in exaggerating lactic acidosis on effort and the effects of impaired carbon dioxide transport the latter requires arterial alkalosis to prevent tissue acidosis These mechanisms all favor hyperventilation and thereby contribute to dyspnea In addition the changes in the subdivisions of the lung volume indicative of some degree of congestion and/or edema point to a pulmonary factor in the genesis of the exertional dyspnea seen in severe anemia Still another factor favoring exertional dyspnea is the high cardiac output at rest which reduces the cardiac reserve available for exercise None of these mechanisms is altered by the administration of digitalis or other forms of therapy directed at cardiac decompensation they all respond favorably when the anemia is treated successfully

As in the case of dyspnea there is a tendency toward edema formation inherent in anemia even in the absence of congestive failure Older clinicians are aware of this phenomenon and younger physicians still see it occasionally in spite of the earlier diagnosis and more adequate therapy of anemia which obtain today Peters and Eisenman[83] in a thorough analysis of the relation of the level of the plasma protein to edema in various diseases noted that patients with anemia developed edema at levels of plasma protein well above those seen in other diseases associated with edema with the exception of congestive heart failure these authors were unable to explain this phenomenon The change in capillary permeability noted as occurring in animal preparations under anoxic conditions[89 95] may have some bearing on the problem On the other hand it is to be noted that Strauss and Fox[105] in a study entitled Anemia and Water Retention showed that retarded excretion of sodium varying in degree with the severity of anemia was present A tendency of this sort toward salt retention suggests the involvement of some renal mechanism as pointed out by Bradley and Bradley[14] in their discussion of the occurrence of markedly reduced renal flow in anemia Many years ago Rowntree and Fitz[94] caused salt retention in animals by inducing renal vascular stasis It is clear that edema may occur in anemia even in the absence of congestive heart failure The edema of anemia like the dyspnea of anemia disappears when the anemia is cured When exertional dyspnea edema and the commonly occurring hepatomegaly of severe anemia occur together the diagnosis of myocardial insufficiency suggests itself However the absence of venous engorgement and more especially of orthopnea contradict that diagnosis Absence of cyanosis is not helpful for as Lundsgaard and Van Slyke[77] showed it is impossible for cyanosis to develop under any circumstances in patients with less than five grams per cent of hemoglobin

V Summary

The cardiac and respiratory adjustments in chronic anemia and their clinical manifestations have been reviewed When the oxygen carrying capacity of the blood is diminished an adequate supply of oxygen to the tissues is maintained by an increased cardiac output an increased velocity of blood flow and a relatively more complete abstraction of the oxygen from the blood as it passes through the capillaries With the increased blood flow the average peripheral resistance is decreased but the state of the small blood vessels is not uniform everywhere the blood flow in the hands and kidneys for instance may be reduced while that of other parts of the body is increased The total oxygen consumption of the body in anemia is not strikingly altered The blood volume generally is slightly reduced but the plasma volume is normal

The deviations from the normal values vary from patient to patient but generally are definite when the hemoglobin values are less than 50 per cent and are greatest at the lowest levels of hemoglobin concentration

The close interrelationship between the cardiovascular and respiratory systems is exemplified by the coincident changes in the respiratory system in anemia The rate and depth of respiration often are increased together with a lowering in the vital capacity and its subdivisions the reserve and complemental air volumes The resid

ual air is somewhat increased These deviations from the normal are similar to those observed in pulmonary congestion or edema and denote a loss of elasticity and expansibility favoring the occurrence of exertional dyspnea The arterial blood saturation is usually normal at rest but, during exertion a significant lowering becomes apparent

The importance of hemoglobin in the transport of carbon dioxide is reviewed the decreased availability of hemoglobin as a buffer in carbon dioxide transport in anemia is compensated by the increased ventilation of the blood in the lungs rendering the arterial blood somewhat alkalotic The red cells also play an important role in regard to the respiratory enzyme carbonic anhydrase In the anemias due to blood loss malnutrition chronic infection uremia or leukemia the blood carbonic anhydrase activity is parallel to the decrease in hemoglobin level leading to a deficiency not only of oxygen carrying capacity but also a decreased ability to absorb carbon dioxide from the tissues and to release it in the lungs The following factors many of which are closely interrelated are operative in the production of dyspnea in anemic patients the increased respiratory minute volume the decreased vital capacity and its subdivisions the abnormalities in carbon dioxide transport and dissociation the reduced arterial oxygen capacity and the decreased blood oxygen saturation during effort and the frequently observed elevated blood lactic acid values

The symptoms and signs exhibited by anemic patients including palpitation and breathlessness on exertion tachycardia cardiac dilatation and hypertrophy are described In addition to an apical systolic murmur other systolic and diastolic murmurs are occasionally heard The arterial blood pressure is frequently lowered in anemia the venous pressure is generally within the limits of normal Electrocardiographic abnormalities occur in approximately one quarter of anemic patients but are minor and not specific in character

The occurrence of angina pectoris congestive failure and intermittent claudication in some patients with the development of anemia and disappearance of these conditions as the anemia is alleviated is discussed with particular reference to the underlying physiologic mechanisms

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MYELOFIBROSIS ASSOCIATED WITH TUBERCULOSIS

A Report of Four Cases

By Howard W Craig M D Howard L Alt M D
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Introduction and Review of the Literature

Myelofibrosis is a disease in which the normal blood forming elements of the bone marrow are replaced by fibrous tissue with compensatory extra medullary hemopoiesis arising in other organs of the reticulo-endothelial system Clinically it is characterized by pains in the long bones back or abdomen progressive weakness pallor and subsequent loss of weight The spleen liver and sometimes the lymph nodes become enlarged and a refractory anemia of the myelophthisic type develops Immature leukocytes with or without an increase in total count and frequently the platelet count is either decreased or increased Bone marrow studies show hypoplasia usually an increase in the megakaryocytes and eventually fibrosis The onset of the disease is insidious and it may last from a few months to years depending upon the degree of compensation but the eventual outcome is fatal

Myelofibrosis was first described by Heuck[1] in 1879 and since that time approximately 100 cases have been reported in the literature under a great variety of titles the most common of these are leuco-erythroblastic anemia chronic non leukemic myelosis [3] and myelofibrosis associated with a leukemoid blood picture [4] The name myelofibrosis was first applied to this disease in 1937 by Mettier and Rusk [4] In 1944 Erf and Herbut[5] contributed materially to this subject by their extensive review of the literature and classification of myelofibrosis as either a primary disease or a disease secondary to such conditions as benzene or fluorine poisoning irradiation and malignant extension No mention is made of the possible etiologic role of tuberculosis although such a relationship had been previously suggested Among 91 cases of myelofibrosis reported in the literature there were 7 cases with definite evidence of active tuberculosis A summary of the findings in the 7 cases is recorded in table 1 The first American to report a case of myelofibrosis was Donhauser[6] (case A) in 1908 His case was found to have an active tuberculosis involving the mesenteric lymph nodes and he proposed a toxic etiology for the primary marrow disease One of the 5 cases reported by Dyke[7] (case B) in 1924 had miliary tuberculosis the remaining 4 had other bacterial diseases with bone marrow involvement and he suggested a disseminated bacteremia as an etiologic factor in this disease Krasso and Nothnagel[8] (case C) in 1925 found atypical tuberculous lesions in their case which they believed were caused by avian tubercu

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losis Emile Weil, Chevallier and See[9] (case D) in 1933 proposed the possibility of a tuberculous etiology for this disease and in 1935 Hugonot and Sohier[10] reported a case (case E) of myelofibrosis associated with tuberculosis and agreed with this etiologic relationship Stone and Woodman[11] (case F) in 1938 reported a case of myelofibrosis with tuberculosis They point out the frequency in which tuberculous lesions are found in diseases of the reticulo-endothelial system Carpenter and Flory[3b] in 1941 concluded that the tuberculosis in their case (case G) was a coincidental terminal disease

The purpose of this paper is to review in detail the clinical picture and autopsy findings of 4 cases of myelofibrosis associated with generalized tuberculosis and to discuss the pathogenesis diagnosis and treatment of this syndrome We have observed 5 cases with idiopathic myelofibrosis which will not be reviewed in detail

TABLE 1 —*Summary of Cases from the Literature of Myelofibrosis Associated with Tuberculosis*

Case	History		Physical Findings				Laboratory Examination			Clinical Course	Autopsy				
	Age Sex	Weight loss	Fever	Splenomegaly	Hepatomegaly	Lymphadenopathy	Anemia	Leukemoid reaction	Thrombocytes	Duration of illness	Tuberculosis: Focal	Tuberculosis: Miliary	Extramedullary hemopoiesis	Marrow fibrosis	Fibrosis of other organs
										months					
A	M 58	++	++	+++	+	–	++	+	?	4	–	+	+	++	+++
B	M 34	?	++	?	?	++	+	?	?	6	–	+	?	+	?
C	M 45	++	+++	++	+	+	+	+	?	24	–	+	+	++	?
D	? ?	?	?	+	+	+	++	++	?	?	+	?	+	++	+
E	M 64	++	+++	+++	++	+	++	+	?	26	–	+	+	+	+
F	F 43	++	?	++	+	+	++	+++	?	12	+	–	+	++	+
G	M 33	++	+++	++	++	+	++	+++	+++	40	–	+	+	+++	++

? = not recorded

in this communication but serve for general comparison with the tuberculous group

REPORT OF CASES OF MYELOFIBROSIS ASSOCIATED WITH TUBERCULOSIS*

CASE 1

C B a 39 year old housewife was admitted to the hospital June 7 1941 complaining of pallor weakness dizziness a skin rash and fever

Family history Irrelevant

Past history Amenorrhea had been present for nine years She had pneumonia at 36 years of age and again at 38 She was occasionally mildly jaundiced and bruised very easily during the last few years

Present illness The pallor weakness and dizziness had been present for the previous thirteen years accompanying episodes of unexplained anemia These episodes recurred with increasing frequency and persisted during the previous eighteen months There was no bleeding from any of the orifices She failed

* Only contributory clinical and pathologic findings are described

to respond to either liver or iron and received whole blood several times Following a splenectomy and subsequent phlebitis she had a daily afternoon temperature elevation occasionally reaching 101 F The skin rash started on the left leg three weeks before hospital entry it then extended to the right leg and both arms The rash was maculopapular at first later indurated and finally tender

Physical examination The pulse was 110 per minute blood pressure 116/76 respirations 24 per minute temperature 101 6 F and weight 99 pounds She appeared somewhat emaciated and chronically ill Over the hands elbows and knees the skin had a dusky appearance An eruption over the legs and arms varied from small red macules papules to reddish purple eczematoid tender indurated nodular lesions distributed mainly over the extensor surfaces The sclerae were white and the mucous membranes pale There were a few small cervical axillary and inguinal lymph nodes palpable Occasional coarse rales were heard over the base of the left lung posteriorly with slight dullness in this same region upon per

TABLE 2 —*Representative Peripheral Blood Pictures from the Cases of Myelofibrosis with Tuberculosis*

Case	Date	Erythrocytes (mill/cu mm)	Hemoglobin (Gm/100 cc)	Leukocytes (per cu mm)	Thrombocytes (per cu mm)	Myeloblasts	Promyelocytes	Neutrophilic myelocytes	Metamyelocytes	Band	Segmented	Eosinophils	Basophils	Lymphocytes	Monocytes	Blast cells	Normoblasts/100 WBC
C B	1/31/40	1 58	6 0	3 400						44	29	2		25			2
	4/23/40	1 59	6 0	9 000							54	4		4	2		53
	6/ 7/41	2 14	7 0	6 050	138 000			2	4	16	45			33			36
W M	11/10/43	2 40	8 5	8 400													
	12/20/43	2 00	5 8	11 800	251 320	5		6	6	29	18	2		30	4		6
	3/13/44	2 6	7 5	3 500	Dec	4				30	18		2	44	2		
W D	1/ 3/46	4 42	13 2	2 850						18	61			16	5		
	2/ 1/46	4 70	14 0	4 700	247 000				2	27	33			25		13	
	5/16/46	3 93	10 5	1 700	133 350					48	32			20			
	6/ 1/46	3 50	9 5	2 100	08 320				3	13	6			8	9		–
M B	10/19/44	3 80	8 1	17 600					3	5	59			28	5		
	6/30/45	1 90	4 35	60 000	500 000			10	8		14			22	42	4	31
	8/13/45	3 50	8 7	115 000	00 000	60	13	6	8	4	5			2		–	

cussion There was a tachycardia and a coarse systolic murmur was audible over the entire precordial region The abdomen was soft and not tender the liver was not palpable

Laboratory examination The urinalysis showed no sugar and a trace of albumin The sediment contained 3-5 erythrocytes per high power field and on one occasion hyalin and granular casts The Wassermann and Kahn tests were negative A fractional gastric analysis showed 48 units of free HCl and o units of total acid The BMR was plus 6 per cent The Van den Berg test was 0 8 mg per cent and the icterus index was 9 units Other blood chemistry studies were normal A total of ten stool examinations for blood were found negative Agglutination tests for typhoid paratyphoid dysentery and brucella were negative Sputum cultures revealed no acid fast bacilli but many gram positive diplococci Blood cultures and fecal cultures were negative X ray examination of the chest was normal on 6/[illegible]/41 but one week later there was evidence of fluid at the base of the left lung Hematologic studies (table 2) showed a pronounced normocytic normochromic anemia with numerous nucleated erythrocytes and a few immature granulocytes in the peripheral blood There was a moderate variation in the size and shape of the erythrocytes The reticulocyte count was 1 3 per cent and the platelets were reduced to the lower limit of normal The bone marrow (table 3) was hypoplastic and revealed a maturation arrest of the erythrocytic series and an increase in the number of megakaryocytes The red cell fragility te

was normal on two occasions The spleen surgically removed on 8/28/40 was reported essentially nor mal A skin biopsy of a nodule from the left arm on 6/10/41 was diagnosed as possible erythema nodosa Acid fast organisms were found in sections made from paraffin blocks containing sediment from pleural fluid A guinea pig inoculated with pleural fluid was found to have epitheloid tubercles of the lungs liver and spleen

Clinical course The patient had a very stormy course throughout her hospital stay with spiking daily afternoon temperature elevations sometimes to 106 F preceded by chills She became dyspneic irra tional and very restless and expired on 7/3/41

Necropsy

Thoracic cavity The left pleural cavity contained 1000 cc of bright red fluid Both lung bases were adherent to the diaphragm and mediastinum by firm fibrous adhesions The pleural surfaces were studded with pin head sized grey raised tubercles Microscopically the alveoli in the left lung base were filled with a fibrinous exudate The tiny tubercles showed a central necrosis with almost no surrounding cellular reaction

TABLE 3 —*Differential Sternal Marrow Counts on Cases of Myelofibrosis with Tuberculosis*

Case	Date	Total count	Myeloblasts	Promyelocytes	Neutrophils myelocytes	Metamyelocytes	Bands	Segmented	Eosinophils	Basophils	Lymphocytes	Monocytes	Broken cells	Pronormoblasts	Normoblasts (all types)	Megaloblasts	Megakaryocytes	Myeloid/erythroid
C B	3/ 5/40		3		15		20	6			6				44	6	Inc	1/1
W M	12/20/43	115 000	8	1	2	2	6	4			3		2	23	40	9	Dec	1/3
	3/13/44	12 500	31		2	1	12	8			31	2		2	6		Inc (5)	11/1
	3/28/44	6 250	10				4			6	48		2	2	4	14	Inc (6)	4/1
W D	2 1/46	1-6 000	3	3	5	8	20			1	5				55		Inc	1/1
	5/16 46	45 000			8	18	25	1	3	2	5			3	28		Inc	2 4/1
M B	7 9/45		16	15	4	3	4	9		4	10			2	33		Inc	2/1

Heart The greater vessels and epicardium were covered with the same tiny tuber les seen on the lungs Histologic study showed an occasional necrotic focus in the myocardium

Liver The liver was enlarged and an occasional tubercle was seen on the cap ule and cut surface The sections showed thickening of the capsule with increased intercellular connective tissue Nu merous tiny areas of necrosis were seen in the parenchyma and each was surrounded by a zone of im mature blood cells A few small foci of extramedullary hemopoiesis containing occasional mega karyocytes were seen

Pancreas Grossly the pancreas appeared normal but mi roscopic studies revealed pronounced interacinar fibrosis and numerous small necrotic tubercles a few of which were surrounded by im mature blood cells

Lymph nodes The hilar retroperitoneal and abdominal lymph nodes were all grossly enlarged Many showed discrete pin head sized grey tubercles on their cut surfaces Microscopi ally the normal architecture of the glands was completely destroyed and replaced by proliferating granulom atous fibrous tissue occasionally surrounding small foci of extramedullary hemopoiesis A fe hyp rchromatic multi nucleated cells re embling megakaryocytes were seen Many of the sections showed small foci of tub rculous necrosi and a rare Langhan giant cell

Bone marrow The marrow from the sternum and vertebrae app ared dark red and dry Histolog ically the sections showed a fibrous tissue replacement of the marro cavity leaving small island of hemopoietic tissue and no fat The megakaryocytes were increased in number

Bacteriologic examination All the sections were stained by the Ziehl Neelson technic and the necrotic foci found filled with acid fast organisms

Pathologic diagnosis Generalized miliary tuberculosis extramedullary hemopoiesis in the liver and lymph nodes fibrosis of the liver pancreas and bone marrow

CASE 2

W M a 50 year old lawyer was first admitted to the hospital November 1 1943 complaining of anginal pain intermittent claudication and pallor

Family history and past history Irrelevant

Present illness For two years the patient had noted viselike pain in his chest upon exertion During the last thirteen months shortness of breath came with the chest pain both were relieved by rest Simultaneously he developed cramplike pains in the calf of his left leg brought on by walking An electrocardiogram in February 1943 showed no significant change These symptoms became progressively worse and he subsequently noticed that he was becoming fatigued and pal

Physical examination The temperature was 99 6 F blood pressure 120 60 pulse 96 per minute respirations 18 per minute and weight 18 pounds The lymph glands were normal There was no cardiac abnormality The lungs were clear except for inspiratory wheezes over both apices The abdomen was soft and the liver spleen and kidneys were not felt

Laboratory examination The urine was repeatedly negative The Wassermann and Kahn tests were negative The total serum proteins albumin globulin ratio and icterus index were all normal X-ray examination of the chest showed the lung fields to be clear and the heart shadow less than 10 per cent enlarged Roentgenologic examination of the gall bladder and digestive tract revealed no pathology The electrocardiogram showed only a left axis deviation The BMR was minus 5 per cent Repeated blood cultures stool cultures for enteric pathogens agglutination tests for typhoid paratyphoid and a brucellergen skin test were all negative The blood studies (table 2) revealed a moderate normochromic normocytic anemia with immature leukocytes and normoblasts in the peripheral blood The reticulocyte count was normal and the platelets were slightly decreased in number The sternal marrow (table 3) showed an initial hyperplasia with increased erythropoietic activity followed by a marked hypoplasia with an increase in megakaryocytes

Clinical course Seven days after entry into the hospital the patient was seized with excruciating precordial and epigastric pain with tenderness over the gall bladder all of which disappeared two days later During this stay he had a slight fever for the first two days again on the seventh day and again on the tenth eleventh and twelfth days He was discharged November 27 He was next seen December 14 as an outpatient complaining of increased weakness and had a decline in the erythrocyte count He was readmitted to the hospital December 29 for two days and given 400 cc of whole blood He continued under ambulatory care without response and was readmitted to the hospital on February 2, 1944 There was no fever at this time A slight systolic murmur was heard over the apex of the heart The lungs were normal The liver was normal in size and the spleen was felt for the first time extending two fingerbreadths below the left costal margin There was slight generalized lymphadenopathy He was given two transfusions of 500 cc of whole blood each and discharged after twenty four hours On February 17 while the patient was ambulatory the spleen was found to be the same size The liver was now found three fingerbreadths below the right costal margin and the anemia continued to progress

His last hospital entry was February 23 In addition to previous complaints he had painful defecation and gross blood in the stools for ten days His temperature on admission was 100 6 F pulse 68 per minute respirations 20 per minute and weight 164 pounds (a loss of 14 pounds since his first admission) The liver and spleen were palpable as previously noted A proctoscopic examination was done under caudal anesthesia and a diagnosis of ulcerative proctitis was made Four days later an indurated region just outside the sphincter ruptured and spontaneously drained mucopurulent material He was given numerous transfusions of whole blood for profound anemia but failed to maintain a satisfactory hemoglobin and red cell count level The sternal puncture (table 3) on February 28 showed a pronounced hypoplasia of the marrow with a marked increase in the number of megakaryocytes The temperature rose daily to over 100 F and after the seventeenth hospital day to over 104 5 F The highest temperature rise (105 2 F) was recorded on the thirty-eighth hospital day and the patient expired two days later April 2, 1944

Necropsy

Lungs Neither gross nor microscopic miliary nodules were describ d The sections showed em physema and focal hemorrhages surrounded by a few hyperchromatic multi nucleated cells Pleural thickening was also seen

Mediastinum A firm nodular mass measuring 6 by 3 by 2 cm was found in the right mediastinum lying just behind the superior vena cava superior to the root of the right lung and lateral to th arch of the aorta This mass cut with ease revealing a bulging pinkish surface Several grey green areas 1–2 cm in diameter were seen on the cut surface and several small cystic structures contained puslike material Microscopic studies of this mass revealed confluent lymph nodes almost completely replaced by granulomatous tissue There were central zones of caseation and necrosis surrounded with varying numbers of epitheloid cells and lymphocytes all of which were encased in a fibrous tissue structure A few multinucleated cells similar to those described in the lung were seen No Langhan giant cells were pres nt Several large nerve bundles coursed through the dense fibrous tissue

Abdominal cavity Firm fibrous adhesions were found about the gall bladder and the recto-sigmoid portion of the colon

Liver The liver was enlarged (2900 Gm) and small yellowish nodules were s en beneath the capsule and on the cut surface Histologic examination showed the sinusoids distended and filled with blood A fibrous tissue and lymphocytic infiltration was noted about the portal triad Small caseating foci were seen surrounded by a few epitheloid cells lymphocytes and plasma cells An occasional megakaryocyte was seen Pronounced fibrous tissue infiltration and hyalinization in the regions ad jacent to these atypical tubercles were constant findings

Spleen The spleen weighed 550 Gm and several small nodules were palpated beneath the capsule Microscopically small foci of caseous necrosis were surrounded by a few round cells In one of these a single Langhan type giant cell was seen The sinuses were distended and filled with blood and pig ment Small lymph follicles remained Throughout the pulp numerous large cells with large oval or indented hyperchromatic nuclei were found Many resembled megakaryocytes Hemopoiesis was not pronounced but the number of immature myeloid cells indicated the presence of this function The capsule was thickened

Adrenals The gross features of this organ were not unusual but microscopically both the medullary and cortical layers showed focal necrosis and rather extensive dense fibrous tissue replacement and hyalinization

Gastro-intestinal tract The only finding of significance in the gastro intestinal tract was a nodular mass 2 cm above the pectinate line in the rectum The mass was produced by a thickening of the wall of the gut but the mucosa appeared intact over the area Microscopically the wall was found to be almost completely destroyed by a necrotic process which had begun to include the mucosa as well Numerous inflammatory cells were seen at the border of this lesion Many bacteria were seen but no acid fast organisms were demonstrated

Lymph nodes The lymph glands throughout the body v ere enlarged and firm The histologic study re ealed a complete destruction of the normal architecture The capsule was thickened and the stroma increased The sinuses v ere dilated and contained cells Extramedullary hemopoiesis was seen and a few megakaryocytes v ere present Lymphocytes in various stages of development were noted

Bone marrow Specimens were taken from the sternum ribs and vertebrae and all had a dry appear ance The histologic changes included complete alteration of the normal architecture The marrow was hyperemic and there was a great increase in the number of megakaryocytes Eosinophilic debris and young fibrous tissue v ere replacing the normal marrow elements and isolated islands or pockets of myeloid activity were seen One of the rib sections shov ed an area of necrosis and increase in the number of small lymphocytes

Bacteriologic examination All the sections were stained by the Ziehl Neelson technic and the caseous foci found filled with acid fast organisms

Pathologic diagnosis Caseous tuberculoma of the mediastinum miliary tuberculosis involving the liver spleen and bone marrow extramedullary hemopoiesis in the spleen and lymph nodes fibrosis of the bone marrow pleura liver spleen and lymph nodes phlegmonous proctitis

CASE 3

W D a 29 year old male entered the hosp tal on January 2 1946 compla ning of weakn ss back ache intermittent chills and fever with associated nausea and vomiting

Family history Irrelevant

Past history Between 1936 and 1937 the patient was employed by General Electric X ray Corporation and was exposed to considerable x ray radiation and phenol His leukocyte count at that time was 6 750 In December 1943 he had a soft mass 6 cm in diameter in the lo er left side of the neck with two lymph nodes palpable below this Aspiration of the mass was unsuccessful but it disappeared after five x ray treatments with a total of ,50 r He served a tour of military duty in the United States without illness and was discharged in March 1945

Present illness His weakness bachache and a temperature of 99 F were first noti d in April nine months before admission In July the patient had chills and fever rising to 103 F which lasted for about an hour and recurred every four to eight hours for eight days He remained symptom free for three weeks when he had a similar attack also lasting eight days In early October he had a third bout For three months he remained free from chills and fever but was weak On December 31 he had still another attack On each occasion nausea and vomiting appeared at the height of the febrile episod s During this entire period the patient had a weight loss of 25 pounds

Physical examination The temperature was 100 4 F pulse 90 per minute blood pressure 110/70 and weight 128½ pounds The heart and lungs were normal The lymph glands and spleen were not palpable The liver was felt one fingerbreadth below the right costal margin

Laboratory examination Previous to entry studies included tuberculin and brucellergen skin tests agglutination studies for the enteric pathogens and brucella the Da idsohn heterophile aggl tination test x ray of the lumbosacral spine retrograde and intravenous urograms and fluoro cop c examination of the chest and gastro-intestinal tract All of these were negative ith the exception of a small gastric ulcer demonstrated by fluoro copy Proctoscopic and cystoscopic examinations were negati e During hospitalization the urine had a specific gra ity of 1 010 albumin one plus sugar negative 20–40 erythrocytes and occasional leukocytes per high power field Direct and bacterial examinations of the stools were n gative A guinea pig inoculated with urine did not reveal any evidence of tuberculosis The Wassermann and Kahn tests were negative The cephalin flocculation test as two plus in t enty four hours and fo ur plus in forty-eight hours The chest x rays repeatedly showed a few clean-cut calcified depos ts on the left side radiating from the lung root outward The last film was made about two months before death The tuberc lin skin test was again negative and the electrocardiogram was normal The prothrombin time was 56 4 per cent of normal the bromsulfalein showed 12 per cent retention of dye total protein 6 28 to 3 88 Gm albumin 3 94 to 2 15 Gm globulin 2 34 to 1 73 Gm icterus index 5 units and blood urea nitrogen 12 mg The peripheral blood stud es (table 2) showed a slo ly progressive normocytic normochromic anemia and le kopenia A few normoblasts and immature leukocytes were seen The reticulocyte count was 1 8 per cent and the platelets were normal Aspirated sternal marrow (table 3) showed hyperplasia at first followed later by a d stinct hypoplasia with an increase in megakaryocytes No mal rial parasites were found Biopsy st dies of the sternal bone marrow (fig 2) in J ly revealed complete alteration of the normal architecture The myeloid and erythroid elements were gre tly reduced and widely scattered No f tty tissue remained These normal cellular elements were separated by a mode te amount of fibrous t ssue and eosinophilic debris Biopsy material from a retroperitoneal mass at the ame time was composed of large ep theloid cells with pale vesicular cytoplasm Scattered throughout these cells as a fine net ork of fibrous connective tissue with new growing fib oblasts and diffuse small round cell infiltrations more dense in some areas than othe s A few polymorphonuclear cells and an occa ional large multi nucleated cell with characteristics of a Dorothy Reed cell we e seen The diagnos s was an inflammatory process ith m ny characteristics of Hodgkin s dise se Sections of the liver were essentially normal

Clinical course The patient was discharged Jan ry 13 on his fou th afebrile day only to be readmitted to the hospital January 23 with a recurrence of the backache and eakness followed by chills fever nau ea and vom t g Fine moist insp ratory r les were heard th ougho t the chest and a soft systolic murm r was a d ble o er the tricusp d area The liver was four fingerbreadths below the right costal m gin and the spleen could be palp ted subcostally There was no denopathy H s fever subsided March 7 and he was discharged ten days later On March 26 the pat ent was readm tted with another attack

but this time with a severe cough and pain and tenderness in the right flank The liver and spleen were palpable as b fore and there was som question of a palpable mass in the region of the right kidney The fever reached 103 F on the second day but subsid d rapidly and the patient was discharged April 2 Tw nty days later the patient made his last entry into the hospital He weighed 123 pounds The liver was palpable four fingerbreadths below the right costal margin and was tender The spleen was again palpable During this admission biopsies were taken from the sternal marrow (*see* Laboratory Examinations) On July 3 a laparatomy was done and enlarged retrop ritoneal tumor masses grossly resembling Hodgkin s disease or lymphosarcoma were seen Biopsies were taken of these and also of the liver (*see* Laboratory Examinations) Eight x ray treatments were given over the abdominal mass with no improvement His temperature ran a very septic course going to 104 F and showed daily and almost hourly fluctuations He continued to lose weight and b cam progressively wors and on August 19 eight days before death his peripheral blood picture revealed marked pancytopenia His pleen and liver increa ed

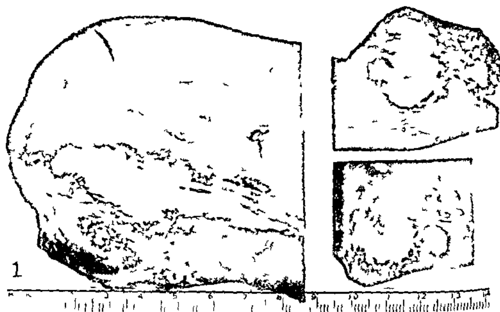


Fig 1 Liver from W D showing large areas of fibrosis

in size and edema became pronounced Treatm nt throughout hi illness included sulfonamides penicillin oral streptomycin (for seven days) atabrine quinine plasmoquin emetine salicylates the x ray treatment mentioned above and four blood transfusions None of these had any effect on the course of the disease

Necropsy

Thoracic cavity Both thora ic cavitie contained about 1100 cc of a clear yellow fluid

Lungs The lungs were adherent to the parietal pleura by a few firm fibrous adhesion and their surfaces were covered with greyish white raised nodules ranging from pin head size to 0 2 cm in di meter The cut surfaces were wet and the same nodules were seen The microscopic appearance of the lung was that of a multitude of tiny anemi infarcts in all the sections The centers of these foci were caseous but there was almost a complete abs nce of the peripheral cellular reaction so common in tuberculosis No Langhan giant cell were observed Many of the surrounding alveoli contained cellular debris and fibrin resembling the consolidation of pneumonia The pleura was thickened

Abdominal cavity The abdominal cavity was filled with clear yellowish fluid There was a large nodular perivertebral retroperitoneal mass in the epigastric region

Liver The liver was enlarged (1650 Gm) The surface was speckled with p n head sized subcapsular greyish white n dules a few larger yellowish nodule and firm white irregular patches (fig

1) The cut surface was similar except that the firm white areas were very tough and extended deeply into the parenchyma mainly about the larger vessels Histologi examination revealed a thickened capsule and distenti on of the sinus s ith blood Scattered throughout the parenchyma were a multi tude of tiny tubercles Many of these contained one or two typical Langhan giant cells A few were surrounded by the typical cellular reaction seen in tub rculo is The large white plaques described at autopsy v ere composed of organized f brous and hyalinized connective tissue and at the borders the connective tissue extended into the parenchyma leaving i lands of li er cells b-hind Foci of lymphocytes were scattered throughout this fibrous tissue An occasional m gakaryocyte and a few small foci of extramedullary hemopoiesis ere seen

Spl en The spleen was enlarged (,oo Gm) and a small acce sory spleen was found Numerous small greyish white nodules were seen beneath the capsule and on the cut surface Firm white irregular plaques on the cut surface ere similar to tho e seen in the li er The sections sho ved a thickened fibrous capsule The sinusoid v ere distended with blood The lymph foll cles v ere completely d s persed and only an occasional small aggregate of lymphocytes could b- fo nd about a essel Large areas of fibrosis were seen but the m st unusual finding was the vid spread seeding of small tubercles as seen in the li er There v as little surro nding cellular reaction though occasional Langhan giant cells were found in the tub-rcles Immatur myeloid and erythroid cells and a moderate number of large cell v ith large irregular den e nuclei resembling m gakaryocytes could be seen d ffusely scat tered throughout the sections

Pancreas (fig 3) Microscopic examination re ealed an increa ed amount of connective tissue and several atypical t bercles similar to tho e previously described

Adre ls The microscop c section sho ved an increase in fibrous tissue and numerous small atypical tubercles

Ret p t l lymph nodes The retroperitoneal mass was an irregular enlarged adherent group of lymph nodes which when sectioned sho ved occasional small yellowish foci Histologic study sho ved a complete obliteration of the normal archite ture ith a greatly incre sed stroma Much chronic gran lomatous tissue as present v ith a fe scattered lymphocytes an occ sional multinuclear Sternberg Reed type of cell and atypical tubercles Ner e bundle were seen encased in the fibrous tiss e

Bon marr w (fig 4) Bone marro v taken f om the sternum rib and verteb ae showed almost com plete obliteration of the marrow cavities by dense fib ous tissue Only a ery few normal myeloid and erythroid foci could be seen Megak ryocytes were prom nent and an occasional atypical tubercle containing acid f st organism was found

Bacte l g c exam nat on All the sect ons were stained by the Ziehl Neelson technic the ca eous foci everywhere and the connecti e tiss e of the liver spleen and lymph nodes v ere filled ith acid fast or ganisms These acid f st org nisms ere cultured on glycerin-egg media inoculated into a series of laboratory an m ls and tested as to streptomyc n sensitiv ty The o g nism wa proven to be a human type of M tuberculosis po sibly of low rulence and sensiti e to streptomycin

Patholog d agnos Diffuse fibros s of the liver spleen lymph nodes adren ls and bone ma row with nteracinar fibrosis of the pancreas extramed llary hemopoiesis in the spleen and liver generalized m li ry tuberculo i

CASE 4

M B 36 y r old hou ewife entered the hospital June 23 945 compla n ng of weakness night sweats he d che chest pa nd a nonprod ctive co gh

Fam ly h tory A si te d ed from tube c lo is

Pa t h tory Irrelevant

Present illness The p ti nt was apparently well nd healthy until fter the normal delivery of a b by Octobe 1 944 T vo d ys postp rtum n enl ged spleen vas found and subsequently a diagno is of B nti s d se s w s m de A pl nectomy wa done No ember 4 1944 The pathologic repo t on the spleen suggested Hodgkin s d e se Follow ng op-ration the p tient gained 35 pounds and re umed her norm l dut e In M y 945 her menstru l period l ted twelve days and she p sed numerous large clots At this t me h c ght cold and her tempe tu e rose to o3 F for few d y She continued







Fig 2 (upper) Photomicrograph of sternal marrow biopsy from W D showing infiltrating fibroblastic tissue cells with dispersion of the blood forming element

Fig 3 (center) Photomicrograph of the pancreas from W D showing interacinar fibrosis

Fig 4 (lower) Photomicrograph of the bone marrow from W D at autopsy showing extensive fibrosis with little remaining hemopoietic tissue

to feel ill and began to lose weight Weakness then became her major complaint and was soon followed by night sweats a persistent headache pain in the chest and back and an irritating nonproductive cough

Physical examination Her temperature was 100 2 F pulse 100 per minute blood pressure 130/72 and respirations 24 per minute A soft systolic murmur was audibl over the base of the heart The lungs were normal The liver was enlarged extending appr ximately five fingerbreadths below the right costal margin A moderate generalized lymphadenopathy was noted

Laboratory examination The urine contained a trace of albumin and 7–10 leukocytes per high po er field The Kahn test was negative and blood chemistry studies including the icterus index were all normal The BMR was plus 20 per cent and the electrocardiogram was normal X ray examinations of the chest were repeatedly normal until a few days b fore death when a diffuse flocculant increase in density was seen involving both lung fields This was most pronounced at the bases and s ggested an acute pulmonary edema The peripheral blood studies (table 2) sho ved a predominance of young myeloid cells with a great increas in the number of megakaryocytes The pathologic s ctions from the spleen were re-examined and reported suggestive of myelogenous leukemia

Clin al cours The treatm nt consisted of repeated blood transfusions The patient grew progressively weaker and continued to los weight Her temperature was high the peaks ranging b tween 104 F and 105 F and there was delirium In the last week of her life ascites and jaundice were present She died September 21 1945

N crop y

Thorac c ca ity Both pleural cavities were obliterated by firm adhesions between the visceral and parietal pleurae

Lung The pleural and cut surfac s of the lungs were studd d with n merous small greyish hite firm nodules varying in size from 0 1 to 0 5 cm On microscopic study tiny areas of focal necrosis were surround d by a zone of immature blood cells and phagocytes Foci of extramed llary hemopoiesis and numerous megakaryocytes were seen

Abd minal ca ity Fi e hundred centimeters of dark straw colored fluid were found in the abdominal cavity The visceral and parietal peritoneum was studded with small greyish hite nodules

Li er The liver was enlarg d (2670 Gm) Th capsule and cut surfaces were covered with small tubercles Histologic study (fig 5) sho ved areas of focal necrosis and infarction s rrounded by zones of hemopoiesis The liver cords were small and surrounded by hyalinized connective tissue which obliterated many of the sinuses Foci of extramedullary hemopoiesis and megakaryocytes were seen

Pancreas Microscopically there w s a pronounced periductile perivascular and p riglandular fibrosi

Ad enals The sections showed fibrous tiss e replacement with separation of the cortical and medul lary cells and microscopic areas of cas ation and foci of extramedullary hemopoiesis

Bon mar ow The cortices of the ribs sternum and vertebra were thickened and the trabeculae prominent The medullary spaces were filled with a dry fibrous like tissue Microscopically the normal marrow was completely d storted (fig 6) Relatively few hemopo etic cells remained and thes were widely d spersed thro gh the connective tiss e filling the marrow spaces The mega k ryocytes appeared increas d in number Necrot c foci were also found

Lymph n d s The med stinal retroperitoneal and perivertebral lymph nodes were enlarged and matted together Micr scopic study of these lymph nodes showed proliferating connective tissue infiltrated w th immature blood cells a d a few meg karyocytes Many of the nodes cont ined focal areas of nec osi

B t i l gi exam nat All of the sections were st ined by the Ziehl Neelson technic and huge numbers of ac d fa t org nisms were fo nd n the areas of n crosis in all the org ns

P th l g c d gn s E tramedullary hemopoiesis in the liver lung spleen and lymph nodes exten ive fibrosis of the li er pleen pancreas dren ls lymph nodes and bone marrow generalized acute diffuse mil ary tuberculosi

Analysis of Cases

A summary of the clinical hematologic and pathologic data of the four cases of myelofibrosis with tuberculosis are recorded in tables 2 3 and 4 We studied W M W D and M B during life and the records of C B several years after death M







FIG 2 (upper) Photomicrograph of a sternal marrow biopsy from W D showing infiltrating fibroblastic tissue cells with dispersion of the blood forming element

FIG 3 (center) Photomicrograph of the pancreas from W D showing interacinar fibrosis

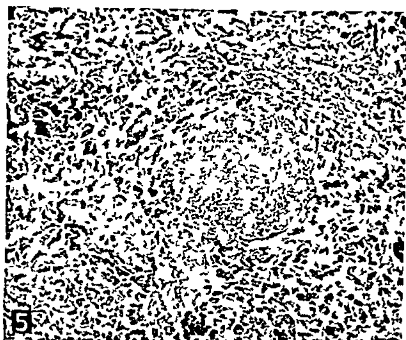
FIG 4 (lower) Photomicrograph of the bone marrow from W D at autopsy showing extensive fibrosis with little remaining hemopoietic tissue

or abdomen fatigue progressive weakness pallor and loss of weight Their *ages* ranged between 29 and 50, averaging 33½ years The *sex distribution* was equal A definite *history of a tuberculous* contact was obtained in M B W D gave a history of cervical adenopathy which was suggestive of tuberculosis

Physical findings All of the patients showed a daily afternoon *fever* with occasional spikes to 104 F During the last few months the daily elevations frequently went above this mark W D had repeated recurrences of chills and fever sometimes two or three a day with afebrile remissions lasting eight to twenty days The *spleen* in each case was moderately enlarged Splenectomies were done on C B and M B early in their illnesses with probable adverse effects The *livers* were markedly enlarged smooth and nontender in three cases In C B the liver was palpable only on deep inspiration Generalized *lymphadenopathy* was moderate in all cases The retroperitoneal lymph nodes were greatly enlarged in W D and were palpable through the abdominal wall as a firm epigastric mass

Laboratory findings A refractory *anemia* was present in all cases The anemia was profound throughout the illnsss of W M and he required frequent transfusions In the remaining cases the anemia was slowly progressive but became terminally pronounced A *leukemoid reaction* characterized by the presence of immature leukocytes and normoblasts occurred in each case Three cases (C B W M and W D) had a progressive *leukopenia* On the other hand M B had a leukocyte count above 100 000 and the differential closely resembled an acute myelocytic leukemia The *platelet* count paralleled the leukocyte count it was reduced to the lower limits of normal in three cases and was well above 500 000 in M B Giant platelets and megakaryocytes were seen in peripheral blood smears of the latter case In each case the *reticulocyte count* was normal The *sternal marrow* aspirations in W M and W D early revealed a hyperplastic marrow Later the marrow became hypoplastic and sternal aspirations then resembled the peripheral blood both in total and differential cell counts The number of megakaryocytes was increased in each case Single marrow aspirations on the two remaining cases showed hypoplasia with an increase in the megakaryocytes A bone marrow biopsy was done on only one patient (W D) The sections revealed a depletion of the normal hemopoietic tissue and fat with beginning fibroblastic replacement and a marked increase in the megakaryocytes One patient (W D) consistently had a low grade hematuria *Stool* examinations for blood were repeatedly negative in all instances Uniform *blood chemistry* studies were not carried out on these patients however W D and M B showed evidence of a slightly decreased liver function The icterus index was normal in each case *Bacteriologic* and *serologic studies* were repeatedly negative in all but C B where acid fast organisms were recovered from fluid aspirated from the chest late in the illness Unfortunately tuberculin skin tests were made in only one case (W D) and were reported negative *Roentgenologic examinations* of the chest revealed evidence of old healed tuberculosis in each case Terminally pleural effusion was found in C B and mottling suggestive of edema in M B Since miliary tubercles were found in the lungs at autopsy serial roentgenograms might have been of diagnostic aid late in the disease

Clinical course All patients ran a continued down hill course with progressive



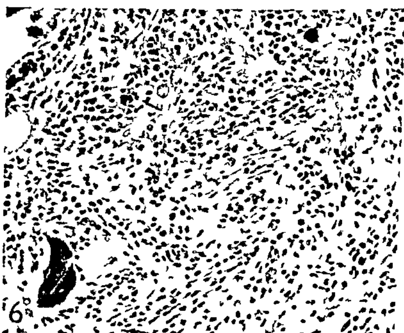


Fig 5 (upper) Photomicrograph of the liver from M B showing miliary tubercle with associated *fibrosis and hyalinization*

Fig 6 (lower) Photomicrograph of the vertebral bone marrow from M B at autopsy showing extensive myelofibrosis

B has been reported[1] previously but is included to make the present study more complete The diagnosis of tuberculosis was made antemortem in C B and was considered but not established in W D

History The *primary complaints* of these cases were pain in the long bones back

TABLE 4—*Summary of Cases*

History		Physical Findings					Laboratory Examination			Sternal marrow				Clinical Course	Autopsy			
										Particle		Biopsy						
No., patient and initials	Age, sex	Weight loss	Fever	Splenomegaly	Hepatomegaly	Lymphadenopathy	Anemia	Leukemoid reaction	Thrombocyte	Hypoplasia	Megakaryocytes	Fibrosis	Megakaryocytes	Duration of illness (months)	Miliary tuberculosis	Extramedullary hemopoiesis	Marrow fibrosis	Fibrosis of other organs
Cases of myelofibrosis associated with tuberculosis																		
1 C B	F 39	++	+++	++[1]	+	+	+++	+	Dec	++	?			18	+	+	++	++
2 W M	M 50	+	+++	++	++	++	++	+	Dec	+++	++			18	+	+	++	++
3 W D	M 29	++	+++	++	+++	+++	++	+	Dec	++	++	+	++	17	+	+	+++	+++
4 M B	F 36	++	+++	++[1]	+++	++	++	+++	Inc	+	++			12	+	+	+++	+++
Cases of idiopathic myelofibrosis																		
5 W H	M 66	++	N	+++	N	+	++	+++	Dec	+	+	++	+	58	—	+	+++	+
6 M S	F 57	++	+	+++	N	N	+	++	Dec	+	++	++	++	14	—	No autopsy		
7 J K	F 53	N	N	+++	+++	N	+	+++	Dec	++	N	+	+	30	—	Patient living		
8 W B	M 77	++	N	+++	+++	+	++	+	Dec	++	++	++	++	27	—	Patient living		
9 L K	F 70	—	N	+	+	N	+	+	N	++	?	++	+	120	—	Patient living		

? = not recorded Dec = decreased Inc = increased N = normal
[1]Splenectomy

weakness loss of weight and fever ending in death C B and M B developed terminal jaundice The *duration of illness* ranged from twelve to eighteen months with an average of 16 2 months

Necropsy findings Generalized caseating *miliary tuberculosis* was found in all of the organs in these cases These lesions were small but were found filled with large numbers of acid fast organisms *Extramedullary hemopoiesis* and an increase in *mega karyocytes* were evident in every instance, most commonly in the spleen lymph nodes and liver The *lymph nodes* in each case were enlarged and the normal cellular elements were replaced by proliferating granulomatous tissue *Bone marrow* from the sternum rib and vertebra in each case was replaced by varying amounts of connective tissue which was confirmed by special staining * The most massive fibrosis was present in W D where it had progressed extensively since the time of biopsy three months before death (figs 2 and 4) *Fibrosis* and *hyalinization* of organs other than the bone marrow particularly of the liver (figs 1 and 5) spleen pleura pancreas (fig 3) and adrenals were prominent in all of our cases

Comparison of the Four Tuberculous Cases with Five Cases of Idiopathic Myelofibrosis

We have observed and analyzed 5 cases of idiopathic myelofibrosis (table 4) which will be reported elsewhere Three are still living and 2 have died These pa tients have many features in common with the tuberculous group namely the splenomegaly, anemia leukemoid blood picture fibrosis in the bone marrow with an increase in megakaryocytes and extramedullary hemopoiesis On the other hand there were certain differences in the two groups The idiopathic cases were in an older age group (53 to 77 years) there was no fever except terminally in one case the spleens were larger and lymphadenopathy was less prominent The average duration of illness in this group at the time of writing was thirty two and one half months as compared to sixteen months in the tuberculous patients There was no evidence of active tuberculosis in the idiopathic group In general the idiopathic cases resembled the tuberculous cases hematologically but the latter group ran a septic course and terminated fatally within a shorter time

Discussion

Pathogenesis The possible role of tuberculosis in the production of myelofibrosis has been considered by previous writers (*see* Review of the Literature) However there is no clean cut evidence in favor of this relationship In a preliminary study of this subject one of us (H W C)[1] proposed that the acid fast organisms found in the atypical tubercles of M B and in other cases reported in the literature may be responsible for the myelofibrosis Furthermore since the atypical tubercles in these cases resembled the lesions produced experimentally with avian tuberculosis it was suggested that the organisms be identified in subsequent investigations This was done in W D where the acid fast bacilli were obtained at autopsy and identified according to the method described by Feldman [13] The organism in th case was found to be a human tubercle bacillus possibly of low virulence definitely sensitive to streptomycin

* Mallory s connective tissu stain

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Cases of myelofibrosis associated with tuberculosis																		
														months				
1 C B	F 39	++	+++	++[1]	+	+	+++	+	Dec	++	?			18	+	+	++	++
2 W M	M 50	+	+++	++	++	++	++	+	Dec	+++	++			18	+	+	++	++
3 W D	M 29	++	+++	++	+++	+++	++	+	Dec	++	++	+	++	17	+	+	+++	+++
4 M B	F 36	++	+++	++[1]	+++	++	++	+++	Inc	+	++			12	+	+	+++	+++
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7 J K	F 53	N	N	+++	+++	N	+	+++	Dec	++	N	+	+	30	–	Patient living		
8 W B	M 77	++	N	+++	+++	+	++	+	Dec	++	++	++	++	27	–	Patient living		
9 L K	F 70	–	N	+	+	N	+	+	N	++	?	++	+	120	–	Patient living		

? = not recorded Dec = decreased Inc = increased N = normal
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* Mallory s connective tissue stain

TABLE 4—Summary of Cases

History		Physical Findings					Laboratory Examination			Sternal marrow				Clinical Course	Autopsy			
										Aspiration		Biopsy						
Name and initials	Age and sex	Weight loss	Fever	Splenomegaly	Hepatomegaly	Lymphadenopathy	Anemia	Leukemoid reaction	Thrombocytes	Hypoplasia	Megakaryocytes	Fibrosis	Megakaryocytes	Duration of illness	Miliary tuberculosis	Extramedullary hematopoiesis	Marrow fibrosis	Fibrosis of other organs
Cases of myelofibrosis associated with tuberculosis																		
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1 C B	F 39	++	+++	++[1]	+	+	+++	+	Dec	++	?			18	+	+	++	++
2 W M	M 50	+	+++	++	++	++	++	+	Dec	+++	++			18	+	+	++	++
3 W D	M 29	++	+++	++	+++	+++	++	+	Dec	++	++	+	++	17	+	+	+++	+++
4 M B	F 36	++	+++	++[1]	+++	++	++	+++	Inc	+	++			12	+	+	+++	+++
Cases of idiopathic myelofibrosis																		
5 W H	M 66	++	N	+++	N	+	++	+++	Dec	+	+	++	+	38	—	+	+++	+
6 M S	F 57	++	+	+++	N	N	+	++	Dec	+	++	++	++	14	—	No autopsy		
7 J K	F 53	N	N	+++	+++	N	+	+++	Dec	++	N	+	+	30	—	Patient living		
8 W B	M 77	++	N	+++	+++	+	++	+	Dec	++	++	++	++	27	—	Patient living		
9 L K	F 70	—	N	+	+	N	+	+	N	++	?	++	+	110	—	Patient living		

? = not recorded Dec = decreased Inc = increased N = normal

[1] Splenectomy

Acute caseating miliary tuberculosis is the name Rich[14] applied to the atypical disease seen in our cases He points out this disease may be overlooked at autopsy or if recognized, may be misinterpreted as avian tuberculosis Rich[14] has experimentally produced the lesions of caseating tuberculosis by injection of large numbers of bacilli into the blood stream of a hypersensitive animal They become widely disseminated and are characterized by tiny caseous foci without epithelioid cells lymphocytes or giant cells the organisms multiply rapidly in these lesions and large numbers are found at autopsy If the animals survive the organisms are reduced and typical tubercles develop Complete healing may take place with or without scar formation Pinner[15] believes that this type of reaction is due to an atypical response of the host Clinically and experimentally this massive dissemination of organisms is characterized by toxemia and spiking temperature elevations

The proliferative reactions in tuberculosis have led to widespread investigations According to Rich [14] the factors influencing fibrosis are as follows (1) virulence of the organism (2) race (3) presence and degree of immunity or hypersensitivity, and (4) resistance of the host Sabin[16] studied the tissue response to various fractions of acid fast organisms She found that an unsaponified higher alcohol derived from the waxes produced a remarkable proliferation of fibroblasts both diffusely and in small clumps Kaufmann[17] described a relatively benign form of tuberculosis involving the lymph nodes which he called attentuated tuberculosis it runs a chronic course with massive enlargement of the glands due to a proliferative reaction There is little or no caseation The organisms are often difficult to demonstrate Pinner[15] describes sarcoidosis as a hematogenous tuberculosis with a productive tissue response and no caseation He believes this is an expression of a high degree of specific resistance as manifested by the benign course the absense of tissue destruction and toxemic symptoms and the efficient destruction of tubercle bacilli shortly after focalization has taken place Such a concept embraces a large field of fibrotic and hyalinized lesions resembling tuberculosis Ewing[18] and L Esperance[19] have strongly supported the concept of a tuberculous etiology of Hodgkin s disease

Arneth [20] Muller [1] Pinner[15] and others [2] occasionally observed hematologic findings in miliary tuberculosis similar to those described in our cases These authors point out that a shift to the left of the granulocytes is one of the heralding features of active progressive tuberculosis This may be accompanied by a leukocytosis or a leukopenia * Leukemoid reactions often accompanied by a leukocytosis and the presence of myeloblasts do occur and are at times difficult to differentiate from leukemia [3] A low grade or moderate anemia is common in pulmonary tuberculosis However Pinner[15] states that marked degrees of anemia (below 75 per cent) are strongly indicative of extrapulmonary involvement or of nontuberculous complications Marrow studies in miliary tuberculosis have shown early hyperplasia of all the hemopoietic tissue and later hypoplasia or aplasia Muller [21] in some of his cases describes marrow of generalized tuberculosis filled with eosinophilic debris

* Recently in our clinic autopsy has been performed on a patient who had miliary tuberculosis associated with a hypoplastic marrow without fibrosis During life she had a leuko-erythroblastic anemia

and sometimes connective tissue fibrils with islands of hyperplastic myelopoietic tissue Further evidence that tuberculosis depresses the marrow is demonstrated by the suppression of leukemia by an active tuberculous infection (Jaffe [4] Heinle and Weir [5] and Ulrich and Parks [6])

Undoubtedly the atypical miliary tuberculosis seen at autopsy in the cases reported here and in the literature represent a terminal dissemmination of the type described by Rich [14] However a review of the clinical course physical findings hematologic and pathologic examinations coupled with the autopsy findings and bacteriologic studies strongly suggest a protracted progressive granulomatous disease of tuberculous origin The degree and extent of fibrosis of the marrow and other organs may be dependent upon factors previously enumerated and upon the length of life of the patient The marrow fibrosis in these cases is considered to be part of a generalized disease

Diagnosis The possible diagnosis of myelofibrosis must be entertained when a patient complaining of bizarre pain weakness and weight loss is found to have splenomegaly hepatomegaly lymphadenopathy and hematologic findings of refractory anemia with a leukemoid reaction Tuberculous involvement is to be thought of if there is in addition a recurrent spiking or persistent unexplained fever

A sternal aspiration in myelofibrosis reveals a hypocellular marrow with an increase in the megakaryocytes The tuberculous case frequently shows an initial marrow hyperplasia followed by hypoplasia This material must be carefully studied for tuberculosis as described by Schleicher [7] A *bone marrow biopsy* must be obtained to confirm the diagnosis of myelofibrosis Lymph node biopsies and splenic punctures may be of assistance These tissues should be cultured inoculated into guinea pigs and examined pathologically for acid fast organisms X ray examination of the chest may reveal evidence of miliary tuberculosis but the absence of findings does not rule out this disease With the proper diagnostic approach it should be possible to make the diagnosis of myelofibrosis with tuberculosis ante mortem in many cases

Treatment Blood transfusions temporarily raise the oxygen carrying power of the blood but have no effect on the course of the disease As is to be expected there is no response of the anemia to liver and iron and sulfonomides and penicillin have no effect on the fever Splenectomy and irradiation are contraindicated as they reduce the compensatory hemopoietic mechanism and probably hasten death Streptomycin offers the only hope in the treatment of this disease Recent reports indicate that certain cases of generalized tuberculosis [9] and tuberculous meningitis [30] are benefited by adequate treatment with streptomycin When the diagnosis is made it would seem logical to give 2 to 3 Gm of streptomycin daily over a prolonged period of time Since the disease is characterized by spontaneous remissions one must be guarded in interpreting therapeutic results

SUMMARY

Myelofibrosis (fibrotic bone marrow and usually an increase in megakaryocytes) is characterized by generalized pains weakness loss of weight enlargement of the liver and spleen and a leuko-erythroblastic anemia

Four cases of myelofibrosis associated with generalized tuberculosis have been reviewed in detail Autopsy examination of the 4 cases revealed acute caseating tuberculosis which was considered to be responsible for the bone marrow and generalized fibrosis observed A similar type of tuberculosis occurred in 7 of 91 cases of myelofibrosis reviewed in the literature The pathogenesis of myelofibrosis associated with tuberculosis is discussed

In the diagnosis of this syndrome attention is called to the importance of obtaining a bone marrow biopsy and making a complete bacteriologic and pathologic study of this tissue for tuberculosis

The 4 tuberculous cases here reported as compared with 5 cases of idiopathic type, are younger, have hyperpyrexia less splenic but greater lymph node enlargement and run a shorter course before death

ACKNOWLEDGMENT

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THE SIGNIFICANCE OF MEGAKARYOCYTES IN THE PERIPHERAL CIRCULATION

By Sir Lionel Whitby

SOME of George Minot s earliest publications were concerned with platelets and megakaryocytes and the significance which should be attached to an increase of the former or the presence of the latter in the circulation In a paper entitled Megakaryocytes in the peripheral circulation Minot (1922) pointed out that an increase in circulating platelets was usual whenever megakaryocytes were present in the peripheral blood with the exception of myelogenous leukemia in which despite megakaryocytes the platelets might still be normal and even decreased Minot s general observation was that whenever megakaryocytes or fragments were found there was usually other evidence of a grave disturbance of marrow function as suggested by the simultaneous presence of immature cells of either the white or red cell series or both This was an early conception of the phenomenon nowadays known sometimes as leuko-erythroblastic anemia or sometimes as a leukemoid blood picture caused by a nonleukemic disease

As to myelogenous leukemia in which primitive leukocytes (and usually red cells) were already present Minot suggested that the appearance of megakaryocytes might be a sign of an acute exacerbation of the disease he observed that when megakaryocytes appeared the blood picture often changed from myelocytic to the terminal myeloblastic predominance

Minot s general deductions were that since neither myeloblasts nor nucleated red cells nor megakaryocytes are found normally in the blood stream then the finding of any or all of these cells pointed to a serious alteration in the mechanism regulating the emergence of cells from the bone marrow into the circulating blood in such cases the marrow was subject to great strain the pathologic process underlying the regulating mechanism was varied since the immature cells occurred in the blood not only when there were structural changes in the marrow as in myelogenous leukemia but also with other changes probably functional as in pneumonia and sepsis

Nowadays one could add a number of other diseases which bring about structural changes in the marrow to Minot s example of myelogenous leukemia An ability to hint at a correct diagnosis in these other diseases which include carcinomatosis and Hodgkin s disease of bone myelomatosis osteosclerosis and myelosclerosis Cooley s anemia and lipomatosis of the bone marrow (Rosenthal and Erf 1943) can make or mar a hematologist who must always be on the alert when he is confronted with a leukemoid blood picture

Minot s suggestion of the significance of megakaryocytes was therefore an observation of fundamental practical value

In a later publication Minot and Buckman (1923) drew attention to the fact that megakaryocytes may share in the hyperplastic process of both leukemia and erythremia (polycythemia vera) and that in the former condition, the megakaryo

From the Cambridge University Medical School Cambridge England

cytes may sometimes appear to be more involved than the leukocytes so that the blood might indeed be flooded with megakaryocytes and their derivatives the platelets For a time the leukemic process might seem to be almost restricted to the megakaryocytes even as frank erythremia or leukemia cause varying degrees of pathologic activity of myeloid or erythroid tissue in conformity with a definite type This paper has sometimes been quoted as suggesting a megakaryocytic type of leukemia but such is a misrepresentation of Minot and Buckman s views which go no further than to say that the disease process appears to be confined to the megakaryocytes When taken in conjunction with Minot s (1922) earlier paper the significance of megakaryocytes in erythremia would seem to be a hint that the dis ease was in process of transition to some complication possibly to the malignant erythro-leukemic form or to the terminal stage of a spent hyperplastic marrow which is becoming sclerosed This last as I shall presently show is the more prob able explanation in view of the frequency with which megakaryocytes and ab normal platelets are found in the blood in myelosclerotic conditions

Minot and Buckman (1925) followed up their first paper by another on The Blood platelets in the Leukemias in which they concluded that the platelet count yielded useful knowledge for guiding treatment and for appraising the state of the leukemic patient They observed that the platelets especially in myelogenous leukemia might be greatly increased or much reduced whereas in the acute leu kemias and the chronic lymphatic type it was usual to find the platelet count below normal They noted that petechiae and hemorrhages were often associated with platelet decrease and that hemorrhages though not petechiae might be found in chronic myelogenous leukemia even when the platelets were greatly increased

These three of Minot s early papers emphasize four important points of which some are nowadays accepted as commonplace though others are not widely known

1 That megakaryocytes in the circulation are an indication of a serious dis turbance of the bone marrow The fact must be taken into account when framing a prognosis

2 That the bone marrow disturbance is commonly leukemic in origin but not always so In the latter case there is frequently a leuko-erythroblastic anemia and the causes of such must be considered

3 That when megakaryocytes appear in the circulation in leukemia or ery themia they indicate of an impending change in the character of the disease

4 That the hemorrhagic manifestations of leukemia are not due entirely to reduction in platelets

Megakaryocytic Leukemia

Boros and Korenyi (1931) described a case which they designated as megakaryo blastic leukemia The case was severely anemic and had a leukocyte count of the order of 200 000 cells per cu mm among which the most primitive cells were described as large mononuclear leukocytes 10–25 μ in size megakaryocytes typical and atypical complete and fragmented were numerous in the blood The clinical course of the disease as well as the postmortem description suggest a diagnosis of

an acute termination of myeloid leukemia Indeed, there can be little doubt that Boros and Korenyi were observing no more than what Minot had described years before—the appearance of megakaryocytes in the circulation in myelogenous leukemia

The literature also contains a number of reports under such descriptive names as chronic nonleukemic myelosis (Hickling, 1937 Carpenter and Flory, 1941) aleukemic megakaryocytic myelosis (Favre et al 1934) and myeloid megakaryocytic hepato splenomegaly (Downey and Nordland 1939) Most of the cases described under these various titles have exhibited a leuko-erythroblastic anemia, with megakaryocytes and their fragments in the peripheral blood (as much as 26 per cent of all nucleated cells in Carpenter and Flory s case) The spleen and liver have been enlarged but seldom the lymph glands Sections of the spleen the bone marrow and even the liver, as well as sites of extramedullary hemopoiesis have shown numerous megakaryocytes Nearly all such reports concern cases of myelofibrosis, and the frequency with which the megakaryocytic phenomenon is prominent in this condition has been well presented and illustrated in the account given by Rosenthal and Erf (1943) of 17 cases of myelofibrosis and one of osteopetrosis (Albers Shonberg disease) The megakaryocytic tissue in the spleen and other organs arises from myeloid metaplasia sometimes the process has been so prominent that authors have put forward the idea of a megakaryocytic leukemia This cannot be accepted on the evidence presented It would seem that myelofibrosis is the fundamental factor in producing this megakaryocytic type of metaplasia whether the underlying cause of the fibrosis be idiopathic or the spent stage of polycythemia or even myeloid leukemia and other invasive conditions (carcinomatosis etc) of the marrow In practice whenever megakaryocytes fragments giant or bizarre forms of platelets or even gross platelet increase are found in the circulation the question of myelofibrosis should be considered Other small practical points can each or severally support the diagnosis These include the evidence afforded by the other features of the blood count by sternal puncture and more especially by sternal biopsy by radiologic examination of the bones with suitable controls of the same age group and, if thought necessary by splenic puncture

The result of sternal puncture is usually disappointing on the positive side This in itself should raise suspicion when the accomplished operator is unable to obtain a satisfactory marrow sample by puncture and especially if the bone feels gritty on puncture The sample usually contains few cellular elements derived from the marrow but either giant platelets or megakaryocytes are highly suggestive In such cases sternal biopsy should be performed With a section, the fibrous nature of the marrow is revealed and the lack of cellularity often associated with numerous megakaryocytes provides a diagnostic picture Radiologic examination of the bones occasionally shows mottled rarefactions or irregular condensations in the cortices which need to be compared with appropriate controls taken at the same time and with the same exposure (Hynes 1940) Splenic puncture sometimes reveals the characteristic myeloid metaplasia with many megakaryocytes

The following is a brief summary of a case recently referred to me for adjudication

by The Ministry of Pensions it illustrates the confusion which may arise in patients who exhibit splenomegaly with leuko erythroblastic anemia—a confusion which becomes more confounded under service conditions when the patient moves from one hospital to another

Report of Case

Sergeant B E P was examined on re-enlistment in 1940 at the age of 38 and placed in Category A1 He had an uneventful service until during the campaign in N W Europe in 1945 he reported sick with vague pain in the left upper abdomen He was found to have a spleen enlarged to 2½ fingers breadth below the costal margin the liver was not enlarged The blood count performed under field conditions was reported as Hb 13 Gm per cent red cells 4 5 million per cu mm leukocytes 6 5 thousand per cu mm with no exact differential though the report stated that 250 cells were abnormal including myeloblasts myelocytes metamyelocytes and some cells of unknown origin there were also a few early and intermediate normoblasts

The man was evacuated from Europe and reinvestigated in England The record of the blood count was Hb 13 8 Gm per cent red cells 4 4 million per cu mm leukocytes 6 2 thousand per cu mm with 3 6 per cent myelocytes 0 4 per cent myeloblasts 0 8 per cent metamyelocytes 2 megakaryocytes and 0 8 normoblasts per 100 leukocytes Investigations included the exclusion of syphilis and glandular fever and a diagnosis of aleukemic leukemia was made A blood count a month later was approximately the same save that many giant platelets were observed At the same time a sternal puncture was performed which showed essentially the same cell content as the blood except for a slightly higher proportion of myelocytes and larger numbers of giant platelets and 2 megakaryocytes per 100 nucleated cells

The patient then had a number of medical boards where he was labelled ? leukemia ? Hodgkin s disease ? Banti s syndrome At one of the boards it was noted (without comment) that the patient was high colored and had a blood pressure of 160/90 He was discharged from the Army shortly afterwards He worked as male nurse for two years and was then re-examined on account of his pensions appeal He stated that he had gone down hill a little but was reasonably well His blood pressure was 180/110 and there was some left ventricular hypertrophy the spleen was enlarged to three fingers breadth below the costal margin and the liver was easily palpable The blood showed no increase in the anemia the leukocyte count was 6 3 thousand per cu mm with 8 5 per cent of abnormal cells of which 0 5 per cent were myeloblasts 2 5 per cent unidentified cells and the remainder either myelocytes or metamyelocytes there were 3 megakaryocytes and 4 normoblasts per 100 leucocytes

Thus during the intervening two years the character of the leuko erythroblastic anemia had not altered significantly nor indeed had the clinical state greatly deteriorated The spleen had become more enlarged the liver had become palpable and the blood pressure had risen The true nature of this man s disease which might have been suspected from the outset by reason of the hematologic findings is amply confirmed by the subsequent history and later records

Most of the points relative to the title of this article which is virtually the title of Minot s original (1922) paper can be extracted from the above case record and expressed as a

Summary

1 Leuko-erythroblastic anemia with leuko-erythroblastosis rather than anemia when associated either with the presence of megakaryocytes or giant platelets in the circulation is very suggestive of the myeloid metaplasia so commonly found with myelofibrosis

2 Associated splenomegaly with subsequent slow progress to hepatomegaly and a tendency to hypertension are confirmatory clinical features

3 Sternal puncture may or may not confirm the diagnosis but if the specimen contains megakaryocytes the fact is highly significant Difficulty in piercing the bone or in obtaining a satisfactory marrow sample are points in favor of a myelofibrosis which should be confirmed by the histologic examination of a trephined specimen

4 Controlled radiologic examination of the bones is sometimes of value in establishing a diagnosis in the idiopathic disease

5 A similar blood picture may occur with polycythemia vera with myeloid leukemia and occasionally with other conditions in which invasion of the bone marrow occurs In polycythemia vera the finding suggests a terminal phase of exhaustion in myeloid leukemia likewise, the megakaryocytic phenomenon is usually an ominous sign of the terminal phase

6 When megakaryocytes are found in the circulation a diagnosis of myelofibrosis should always be considered

This short and simple article which draws attention to some of George Minot s early work brings with it the greetings of the entire staff of The Cambridge University Medical School to a great physician

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FAMILIAL PANMYELOPHTHISIS

Fanconi Syndrome in Adults

By Karl Rohr, M.D.

THE FOLLOWING case histories are of interest because they are the first reports of a familial panmyelopathy of the hypoplastic type occurring in adults. Two brothers were affected.

Case Reports

Case [illegible] Franz [illegible] was born in 19[illegible]. As a boy he was [illegible] because of marked pigmentation. He was healthy apart from bouts of eczema.

In 19[illegible] at the age of 18 he had poliomyelitis with resulting weakness of the abdominal muscles. Anemia was discovered for the first time during this illness, with hemoglobin levels varying between 60 and [illegible] per cent. In April 1945 the hemoglobin was again 60 per cent. In August 1945 the hemoglobin had decreased to 53 per cent and in September to 51 per cent. He was given transfusions and Ferro[illegible] therapy and the hemoglobin rose to [illegible] per cent. When seen early in 1946 after accidental burning of one arm, the hemoglobin had again dropped to 48 per cent and he was transfused. In June of that year his hemoglobin was 65 per cent. He complained of being very tired and developed dyspnea with little exertion. He also complained of severe pains in the tibiae and vertebrae, of [illegible] edema and gingivitis. In November 1946 three teeth were extracted because of stomatitis and this was followed by fever ranging between 38 and [illegible] C. There was profuse bleeding, the hemoglobin declining to 30 per cent and later to [illegible] per cent. Temporary improvement followed transfusions and penicillin therapy. Later that year he had bronchopneumonia and his hemoglobin was found to be only [illegible] per cent. He died in March 1947 at the age of [illegible] years.

Physical examination showed that the form and the size of the head were normal, as were the genitalia. The skin showed a marked grayish pigmentation, especially on the face, forearms and to a lesser extent on the abdomen. Petechiae were seen in the skin and mucous membranes in 1944 and in November 1946 at the time of the teeth extraction, he showed marked pallor, gingivitis, stomatitis and glossitis and there were hemorrhages in the fundi of the eyes. The heart was found to be slightly enlarged and in September 1945 the blood pressure was 13[illegible]. The electrocardiogram was normal at that time but in November 1946 showed signs of myocardial damage.

The urine showed no [illegible] but no porphyrin. Serum bilirubin was 0.3 mg. per cent and phosphates and phosphatase were normal. The Takata Ara reaction was negative, the Weltmann [illegible] band was [illegible] (enlarged) and the serum proteins were 6.8 Gm. per cent.

Hemat[illegible] The course of the anemia has already been [illegible]d and is shown in figure 1. The [illegible] counts were between [illegible] and 3.5 million per cu. mm., later dropping to 1.1 to 1.[illegible] million per cu. mm. and finally to 660,000 per cu. mm. The color index varied from 0.96 to 1.33 but was usually [illegible].

The white blood cells in 19[illegible] were [illegible] to 8,300 per cu. mm. During 1945 the count was about [illegible],000 per cu. mm. at first, later dropping to between [illegible],000 and 4,000 per cu. mm. In November [illegible]946 the count was [illegible] per cu. mm. and finally reached as low as 310 per cu. mm. The polymorphs were 63 per cent at the beginning with [illegible] per cent lymphocytes. This gradually changed so that the polymorphs dropped to [illegible] per cent and then to 34 per cent, the lymphocytes rising to 31 per cent and later to 60 per cent. Monocytes varied between 4 and 10 per cent and the eosinophils between 1 and 3 per cent. The blood smear showed anisocytosis of marked degree throughout the illness, with macrocytosis and microcytosis and poikilocytosis. Polychromasia was marked for a long time. Reticulocytes were [illegible] per cent in June [illegible] but later declined to [illegible] and [illegible] per cent.

The platelets were noted to be diminished in August 1945 and counts during the next year lay between 4,600 and [illegible], with [illegible] finally to [illegible],000 per cu. mm.

From the Medical Department, University of Zurich, Switzerland.

2 Associated splenomegaly with subsequent slow progress to hepatomegaly and a tendency to hypertension are confirmatory clinical features

3 Sternal puncture may or may not confirm the diagnosis but if the specimen contains megakaryocytes the fact is highly significant Difficulty in piercing the bone or in obtaining a satisfactory marrow sample are points in favor of a myelofibrosis which should be confirmed by the histologic examination of a trephined specimen

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THE FOLLOWING case histories are of interest because they are the first reports of a familial panmyelopathy of the hypoplastic type occurring in adults Two brothers were affected

Case Reports

Case 1 Sch Franz was born in 19 1 As a boy he was nicknamed the negro because of marked pigmentation He was healthy apart from bouts of eczema

In 1939 at th age of 18 he had poliomyelitis with resulting weakness of the abdominal musel s Anemia was disco ered for the first tim during this illness with hemoglobin levels varying betwen 60 and 80 per cent In April 1945 the hemoglobin was again 60 per cent In August 1945 the hemoglobin had declined to 53 per cent and in September to 51 per cent He was given transfusions and Ferroredoxin therapy and his hemoglobin rose to 80 per cent When seen early in 1946 after accidental burning of one arm hi hemoglobin had again dropped to 48 per cent and he was transfused In June of that year his hemoglobin was 65 per cent He complained of being very tired and developed dyspnea with little ex ertion He also complained of severe pains in the tibiae and vertebrae slight edema and gingivitis In No ember 1946 three teeth were extracted because of stomatitis and this was followed by f ver ranging between 38 and 40 C there was profuse bleeding the hemoglobin declining to 30 per c nt and later to 24 per cent Temporary improvement followed transfusions and penicillin therapy Later that year he had bronchopneumonia and his hemoglobin was found to be only 10 per cent He died in March 194- at the age of 26 years

Physical exam nation showed that the form and the size of the head were normal as were the geni talia The skin showed a marked greyish pigmentation especially on the face forearms and to a lesser extent on the abdomen Petechiae were seen in the skin and mucous membranes in 1944 and in No ember 1946 at the time of the teeth extraction he showed marked pallor gingivitis stomatitis and glossitis and there were hemorrhages in the fundi of the eyes The heart was fo nd to be slightly enlarged and in September 1945 the blood pressure was 130/ o The electrocardiogram was normal at that tim but in No ember 1946 showed signs of myocardial damage

The urine showed urobilinogen and indican but no porphyrin Serum bilirubin was 0 3 mg per cent and phosphates and phosphatase were normal The Takata Ara reaction was negative the Weltman coagulation band was 0 25 (enlarged) and the serum proteins were 6 8 Gm per cent

H matol g c findings The course of the anemia has already been indicated and is shown in figure 1 The red cell counts initially were betw een 2 7 and 3 5 million per cu mm later dropping to 1 1 to 1 2 million per cu mm and finally to 660 000 per cu mm The color index varied from 0 96 to 1 39 but was usually over 1 2

The hite blood cells in 1939 were 2 -00 to 8 300 per cu mm During 1945 the count was about 4 000 per cu mm at first later dropping to between 2 000 and 4 000 per cu mm In November 1946 th count was only 500 per cu mm and finally reached as low as 310 per cu mm The polymorphs were 63 per cent at the beg nning with 25 per cent lymphocytes This gradually changed so that the polymorphs dropped to 58 per cent and then to 34 per cent the lymphocytes rising to 31 per cent and later to 60 per cent Monocytes varied between 4 and 10 per cent and the eosinophils between 1 and 3 per cent The blood smear showed anisocytos s of marked deg ee throughout the illne s with macrocytosis and microcytosis and poikilocytosis Polychromasia was marked for a long time Reticulocytes were 2 4 per cent in Jun 1946 but later decl ned to 0 3 a d 0 4 per cent

The platelets were noted to be diminished in August 1945 and counts during the next year lay between 4 600 and 32 000 w ith a drop finally to 1 000 per cu mm

From the Medical Departm nt Unive sity of Z rich Switzerland

The bleeding time was 5 minutes and 1 1/2 minutes and the coagulation time was normal on two occasions (5 minutes) The osmotic fragility test showed initial hemolysis in 0 44 per cent NaCl and complete hemolysis in 0 32 per cent NaCl

The sedimentation rate was first found to be high in 1944 Readings by the Westergren method showed results of 60 to 101 in the first hour and 76 to 130 in the second hour except for readings of 30 for the first hour and 60 in the second hour after transfusions had raised the hemoglobin to 30 per cent In the terminal stages of the illness the readings were 1/2 and 1/5 in one and two hours respectively

Bone marrow studies during life are of interest In 1945 smears showed abundant marrow macroblastosis and increase of the metamyelocytes and stab forms In 1946 the marrow showed a good deal of fat hypocellularity with few basophilic erythroblasts and myelocytes and almost no neutrophils but a great increase in the reticular cells of the lymphoid and plasma cell types (fig 2) In 1947 the marrow was even poorer in the normal cell types Lymphoid and plasma cells predominated being seen in groups of 6 or 8 Lymphocytes were also increased in parts a great many fibrocytes with fibril formation were seen and there were also an unusual number of tissue mast cells as many as 4 or 5 per field (fig 3)

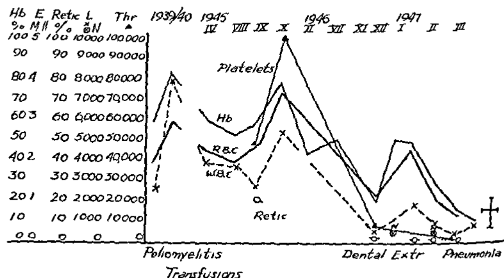


Fig 1 Course of the Blood Counts in Sch Franz

Autopsy report The heart showed dilatation with hypertrophy of the left side and hemorrhages into the endo- and myocardium There was bronchopneumonia at the right lower lobe The liver showed fatty degeneration The brain showed slight bleeding There were extensive hemorrhages into the mucous membranes Generalized hemosiderosis was observed throughout the whole reticulo-endothelial system and in the liver cells Brown iron free pigmentation of the skin was also observed Rudimentary centers of blood formation with development of megakaryocytes were found in the lymph nodes and spleen Areas of chronic inflammation were seen in the suprarenal medullae and in the interstitial tissue of the kidneys

Case 2 Sch Willi The younger brother was born in 1923 and is now 25 years old The course of his illness is shown in figure 4

Past illnesses were whooping cough measles mumps and bronchitis during childhood and appendectomy at the age of 15

The present illness started in November 1943 at the age of 20 with a cold which was followed by pneumonia of the left lower lobe while the patient was in military service Following Cibazol therapy the pyrexia diminished but a low grade fever continued and a high sedimentation rate persisted with readings of 90 mm in the first hour and 105 mm in the second hour (Westergren) The hemoglobin at the onset of the illness was 86 per cent later dropping to 60 per cent

Following blood transfusion therapy the hemoglobin was 56 per cent but later dropped and the anemia became even more severe Th dis as continued to progress with a hem rrhagic tendency always the most prominent feature with e pecially bad bleeding follo ing the extraction of a tooth He continued to run a low grad f ver with temporary bouts of higher pyrexia of unexplained origin Occasional epi sodes of diarrhea which were resistant to therapy occurred In 1945 the patient began to suffer from

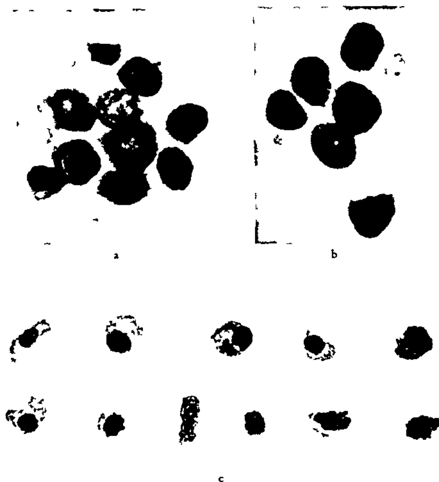


FIG 2 STERNAL PUNCTURE OF SCH FRANZ

(a and b) Microphotographic enlargement (X 1 000) Cl sters of plasma cells with central reticular cells

(c) From the same slide photograph from atercolor picture Smaller basophilic stroma cells (plas mocytic histiocytic and fibrocytes like cells)

v olent pain in the bones and had intercurrent eosinophil c infiltration of the lungs and an attack of epidemic hepatitis At times the e was spontaneous improvement in his condition The dis ase was re sistant to all forms of the apy includ ng sulfonam des penicillin in large doses iron and massi e doses of vitam ns Temporary improvement could be brought about only by transfusions He was given more than 70 transfusions totalling about 20 liters of blood

Because of the failure of all other therapeutic measures splenectomy was carried out in September 1945 Following operation the hemoglobin increased to 70 per cent the bleeding tendency ceased the

general condition improved and the weight increased However a few weeks after operation the anemia again increased with a recurrence of bleeding into the skin Violent pains occurred in the bones of the legs and thighs in the shoulder blades and the vertebral column The skin of the legs gradually became



a



b



c

FIG 3 STERNAL PUNCTURE OF SCH FRANZ

(a and b) Microphotographic enlargement (× 1 000) Various tissue mast cells besides connective tissue and small lymphoid reticular cells

(c) Two isolated mast cells in aplastic anemia photograph from a ater olor picture Note the coarse basophilic granulation and the protoplasmatic ps udopodia

greyish like smoke with dark pigmented spots At the beginning of January 1946 the patient was given high altitude therapy in the Engadine His clinical condition became stationary with frequent violent pains in the legs and pigmentat on of the hands The hemoglobin at this t me was 65 per cent

The clinical condition remain d unchang d p to the end of April 1947 The patient had been able to do some light work for s eral months He often complained of iolent boring pain in the bones of legs

the cervical vertebrae the shoulder blades and the bones of the jaw After preparation by blood trans fusions 11 decayed teeth were extracted without any severe bleeding Only rare bleeding int~ th skin and epistaxis had occurred and there had been no hematuria The temperature had been only slightly elevated except during an influenzal infection The hemoglobin on March 30 1947 was 59 per cent Liver and folic acid therapy were without effect

Physical examinations done at various times during the illness showed striking pigmentation of the skin especially around old scars as well as brownish spots of pigmentation on the mucous membrane of the mouth The skin was delicate and decidedly smoke grey in color particularly on the legs and thighs and to some extent on the arms and body In a few places some darker spots were noticed It was observed that the patient had a slender skull and x rays revealed a thin skull with a small sella turcica The structure of the body was somewhat asthenic and gave the impression of being slightly infantile There were few hairs on the body with hardly any beard growth and feminine genital hair distribution (fig 5) He was found to be intellectually normal No abnormalities were found by x ray in the pelvic bone femur or humerus

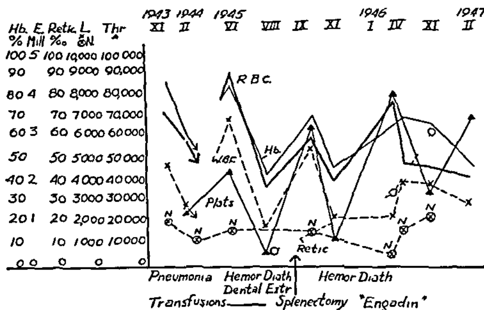


Fig 4 Course of the Blood Counts in Sch Willi

The urine gave a slightly positive test for urobilin and occasionally showed a few isolated red cells Free hydrochloric acid was present in the stomach The stools contained increased amounts of fats but no increase of urobilin X rays showed the stomach and intestines to be normal The electrocardiogram showed deflection of the T wave in the second lead but was otherwis normal The basal m tabolic rate was +4 per cent

Blood chemistry showed total proteins varying from 7 0 to 7 8 gm p r 100 cc cholesterol 147 to 173 mg per 100 cc serum iron 1,2-225 gamma per 100 cc calcium 9 5 mg per 100 cc nonprotein nitrogen 27 mg per 100 cc the uric acid 4 2 mg per cent and the bilirubin 0 3 mg per cent except during the attack of ep demic hepatitis when it rose to 6 2 mg per cent The Takata Ara reaction was negative and the Weltman coagulation band was 0 2 (enlarged)

The Wassermann Pirquet and Mantoux reactions were neg tive and repeated blood cultures were also negative

H matolog fin li g The hemoglobin varied between 59 and 65 per cent except after transfusions and a rise to 70 per cent following splenectomy The red cell count varied from 2 4 to 3 0 million p r cu mm

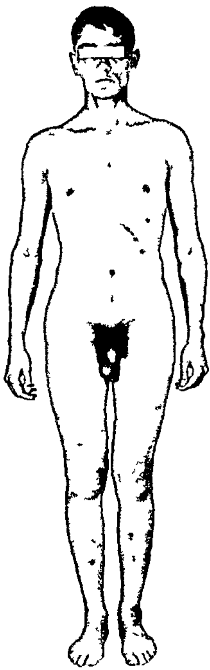


Fig 5 Sch Willi

Note the slight infantile aspect the feminine hair growth and the pigmentation of the skin especially on the legs Status after splenectomy

and the color index from 1 0 to 1 3 The white cell count showed leukopenia 2 800 to 4 500 except after transfusions and after splenectomy when it rose to 5 500 Neutrophils were 39 5 to 45 5 per cent co-

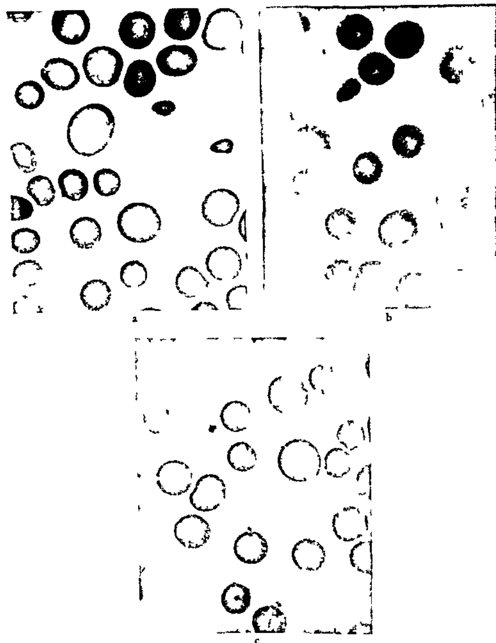


Fig 6 Blood Picture of Sch Willi

Microphotograph c enlargem nt (× 1 000) Note the enormously developed anisocytos s in (a) macrocytic and microcytic (schistocytes) forms and in (b and c) the target cells

sinophils 0 5 to 5 0 per cent basophils 0 0 to 0 5 p r cent monocytes 9 to 12 p r cent lymphocytes 40 to 57 per cent with a rise to 63 p r cent following splenectomy and 1 5 p r cent plasma cells we e seen on one occasion The platelets w re markedly reduced 25 000 to 46 000 except immediately after splenec tomy when they ere 66 000 The reticulocytes were 1 1 to 2 3 p r cent Blood smears (*fig* 6) showed

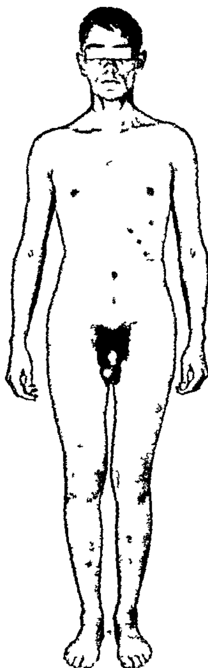


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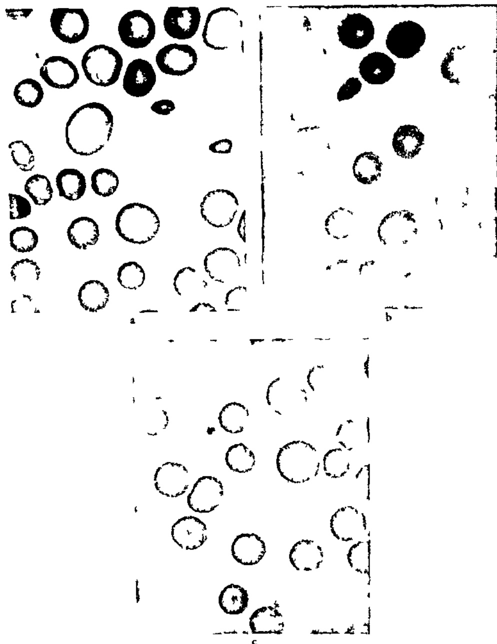


Fig 6 Blood Picture of Sch Willi

Microphotographic enlargem nt (× 1 000) Note the enormously developed anisocytosis in (a) macrocytic and m crocytic (schistocytes) forms and in (b and c) the target cells

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aniso ytosis with very large and very small cells poikilo ytosis polychromasia and even before splenec tomy erythroblasts and Howell Jolly bodies were present

The bleeding time was 6 minutes with a temporary rise to 90 minutes in 1945 Th proth omb n time was 40 p r cent later 100 p r cent The coagulation time was 1 to 12 minutes The osmotic fragility test showed initial h molysis in 0 5 p r c nt NaCl and complete hemolysis in 0 1 p r cent NaCl

Th sedimentation rate was persistently elevated Early in the diseas it was 45 mm in the first hour and 75 mm in the s cond hour by the Westergren m thod rising to 70 mm and 110 mm in th first and second hours respectively except for readings of 8 and 15 respectively during a remission in the anemia In April 1946 the readings varied from 18/39 to 26/45 and in March 1947 the result was 28/47

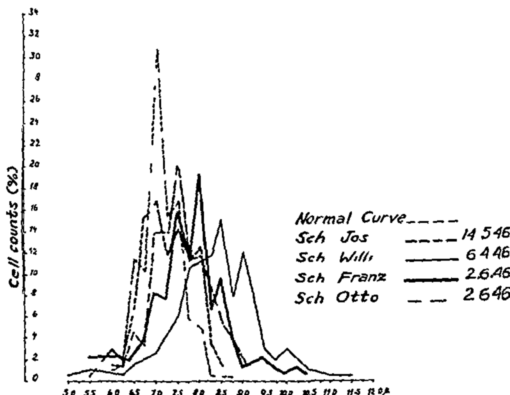


Fig 7 Pri e Jones Curve of the Four Brothers

Note in comparison with the normal curve the deflation and broadening of the curve especially toward the right a tendency which is strongly marked in the still living patient Sch Willi in the deceased Sch Franz less remarkable and scarcely noticeable in the healthy brothers

Hist logic findings The *spl en* histologically show ed mod rat thickening of the capsule and trabeculae as well as thickening and hyalini ation of the intima of the follicular arteries The pulp contained copious amounts of red cells with a moderate numb r of lymphocytes some neutrophils and eosinophils numerous hemosiderin-containing pulp cells and ome plasma cells The venous sinuses ere also enlarged A diagnosis of chronic splenomegaly and hem sidero is was made

Sections of a *g owth excised f m the subcut f the l g* showed perivascula lymphatic infiltrations with som isolated polymorphs Nume ou hemo derin-containing cells were found in the connecti e tissue as well as in the corium

Sections of the bone marrow also showed nume ous hemosiderin containing macrophages and a few lymph follicles The bone marro w as als studied by sternal puncture do e s en times during the course of the disease The corticalis was m derately hard At first the marrow was rather abundant but later became scarce The most consistent finding was an increase of the immature myelocytes and of big ba

sophilic erythroblasts while megakaryocytes were se n rarely In several punctures many reticular cells especially larger and smaller plasm cytic forms were present On one occasion tissue mast cells were remarkably abundant and they were seen in a few other instances especially in places where the bone marrow was thick

Fam y History The maternal grandparents d ed at 70 and 84 years of ag both of cancer of the stomach The father is 56 years old The mother is 53 years old and suffers from mild hypertension but is otherwise well Of 8 paternal uncles and aunts on has tuberculo is and 2 suffer from chronic polyarthritis Two brothers are living and well There were twin sisters one of whom was stillborn and the other died one hour after birth

Of 40 relatives examined only 2 showed hematol gic abnormalities In 2 otherwise healthy brothers with hemc globins of 100 and 102 per cent and red cell counts of 4 8 and 5.0 million per cu mm respec tively the Price Jones curves showed a tendency to widening of the base both to the macro- and microcytic sides (fig 7) Their reticulocytes sometimes rose to 2 0 and 2 4 per cent and the serum iron concen trations were 140 and 165 gamma per 100 cc Osmotic fragility tests showed initial hemolysis in 0 48 and 0 46 per cent NaCl respectively and complete hemolysis in 0 30 per cent NaCl in both

The second patient and the parents were Rh positive

Discussion

One of the notable features of the disease in these two brothers lies in their strikingly analogous clinical symptomatology The features common to both cases may be listed as follows

1 *Age of onset of symptoms* In the case of the elder brother symptoms began when he was 24 years old although signs were already present five years earlier The younger brother became ill at the age of 20

2 *Pigmentation* In both patients an abnormal pigmentation of the skin at tracted attention showing sometimes a brown sometimes a more smoky grey color Pigmentation was present in one brother even before other manifestations of the disease appeared and in the other patient the degree of pigmentation was greater than could be accounted for by the hemorrhagic diathesis or by the nu merous blood transfusions

3 *Hemosiderosis* In both cases histologic examination revealed an abnormally marked hemosiderosis in the reticulo-endothelial system

4 *Pain in the bones* Both patients complained at times of violent pain in the bones

5 *Panhemocytopenia* In both cases the entire bone marrow was affected from the very beginning with resultant anemia leukopenia and thrombocytopenia

6 *Hematologic findings* Both patients had a hyperchromic type of anemia with a color index between 1 1 and 1 4 The erythrocytes revealed unusually marked anisocytosis with large macrocytes and some abnormally small microcytes (so called schistocytes) Furthermore in both cases there was a tendency to poikilocytosis occasional target cell formation and to an abnormal amount of polychromasia The number of reticulocytes was almost constantly above normal in both patients The serum bilirubin was normal the Takata Ara test negative and the Weltman coagulation band enlarged and the Wassermann test negative

7 *The morphology of the bone marrow* At the beginning of the illness only the signs of maturation arrest were apparent Later hypoplasia of the marrow paren chyma appeared which progressed to almost complete aplasia of the marrow in the patient who died Moreover in both cases striking changes were present in the

stroma There was marked increase of small as well as larger forms of plasmocytic reticular cells (plasmocytosis) constant increase of the fibrocytes (fibrosis) and in addition unusually exuberant growth of the so called tissue mast cells (mastocytosis) with as many as 4 to 5 such cells per field in some areas

Additonal features of the disease are as follows (a) The younger still living patient showed certain signs not observed in his brother, namely slight infantilism with deficient hair growth, microcephaly a small hypophysis and hypogenitalism (b) In one patient the osmotic fragility of the red cells was increased at the beginning of the illness, while it was normal at the beginning of the illness of the other (c) In one patient a few Howell Jolly bodies and erythroblasts were seen in the peripheral blood even before splenectomy (d) The level of serum iron was continually high in one patient but was not determined in the other (e) There have been no previous reports in the literature of the occurrence in adults of a similar familial form of panhemocytopenia accompanied by such striking pigmentation Many cases of familial anemia agranulocytosis and panmyelophthisis have been reported especially by Gaennslen and Huber [1] However the clinical picture of the two patients reported here seems to bear more resemblance to the constitutional panmyelopathy of children described first by Fanconi in 1927 and known as anemia perniciosiformis constitutionalis or the Fanconi syndrome This disease has also been described by Uehlinger [3] Zellweger and Zollinger[4] and by Dameshek and associates [5] The condition is characterized by a refractory macrocytic anemia with leukopenia and thrombopenia, brown pigmentation of the skin microcephaly atrophy of the testes and a tendency to deformities of the skeleton

Hematologically we are apparently dealing with the same anomaly in the patients reported here Furthermore as reported in the disease in children these patients showed pigmentation of the skin due apparently chiefly to hemosiderosis The infantile features were less pronounced here though they could be seen distinctly in one of the patients The less pronounced degree of these changes seems to be connected with the relatively late development of the disease which set in after the completion of puberty in both patients

Unlike the known aplastic anemias which are either normochromic or show a tendency to macrocytosis it is of considerable interest to find in these patients an unusually marked anisocytosis with on the one hand very large macrocytes and on the other hand very small microcytes (so called schistocytes) as well as poikilocytosis and target cell formation The reticulocytes were increased up to 2 0 to 3 0 per cent whereas they are usually lacking in typical cases of aplastic anemia Although there was little or no increase of bilirubin in the serum and the urobilin elimination in the urine was insignificant there were various other factors which indicated pathological hemoglobin metabolism One indication was increased hemolysis suggested by the high concentration of serum iron the abnormal osmotic fragility of the erythrocytes and the number of reticulocytes Another was the pathologic iron storage throughout the reticulo-endothelial system as indicated by the hemosiderosis of the various organs * The increase of

* This may have been due at least in part to the effects of multiple transfusion it is curious that exogenous hemochromatosis seems to develop much more extensively in cases of hypoplastic anemia than in some other cases of anemia given numerous transfusions *Editor*

hemolysis might be explained by assuming that a more exact balance of hemoglobin metabolism existed

The pathologic functioning of the reticulo-endothelial system in the two patients studied manifested itself not only in the generalized hemosiderosis but also in changes in the bone marrow As mentioned above the changes in the reticulum and in the stroma of the marrow were especially remarkable consisting of marked growth of the reticular cells especially of the large and small plasmocytes of the tissue mast cells and of the fibrocytes These pathologic changes can be summed up with the designation reticulo-fibrosis of the bone marrow The changes in the stroma seem to represent the primary disturbance the first changes being plasmocytosis and mastocytosis This results in maturation arrest of the normal marrow parenchyma which follows as the next stage of the process With the evolution of the disease there ensues a kind of cicatrization process an increase of the fibrosis with a gradual destruction of myeloid tissue and marrow atrophy is a still later stage of the process

At present no definite answer can be given to the question of the physio pathologic importance of the enormous increase of the plasmocytes and mastocytes However it is known that both cellular forms should be classified in the reticulo-histiocytic system and that they belong to the so-called active mesenchyma The plasma cells undoubtedly play an important part in the formation of globulin particularly of gamma globulin and hence in the development of antibodies Thus a relationship between plasma cells and certain immunity reactions appears to be important On the other hand the mastocytes which show a genetic relation to heparin and amyloid presumably have some connection with anaphylactic processes * It is theoretically possible that these particular plasmocytic and mastocytic changes of the bone marrow are an expression of an *anaphylactic allergic process* of the bone marrow In the light of these facts it is noteworthy that in both patients the whole clinical picture developed in connection with an infectious disease (poliomyelitis and pneumonia respectively) Such a pathologic reaction of the reticulo-histiocytic system not only explains the primary reaction of the stroma of the bone marrow with a tendency to fibrosis of the marrow but also accounts for the abnormal blood picture *

Other pathologic conditions of the reticulum or mesenchyma are known to be accompanied by even greater disturbances of the blood picture This is true especially of osteosclerosis and osteomyelosclerosis where the principal disturbances originate in the osteogenic reticulum and in Cooley s anemia where it is the disturbance of the myelogenic reticulum which seems to be chiefly responsible for the disturbances in the formation of blood In these blood diseases similar morphologic changes of the erythrocytes are found namely marked aniso micro macro- poikilocytosis and target cells These changes are much more pronounced in Cooley s anemia In both these diseases there is not only a disturbance in the formation of blood but also a disturbance in the development of the bones One

We have found tissue mast cells in the bone marrow in but a dozen cases and only in hypoplastic and aplastic anemias of various etiology (benzol poisoning le kemia myeloma infections and in idiopathic forms) [6]

disturbance is not the consequence of the other but pathologic changes occur in both organs from the beginning In Cooley s anemia however the pathologic blood formation is more striking and in osteosclerosis the pathologic bone formation dominates the clinical picture

It is not difficult to explain generalized hemosiderosis and pigmentation of the skin and mucous membranes as a consequence of pathologic functioning of the reticulo histiocytic system Abnormal hemolysins or agglutinins were not detectable in the two patients reported here The parents and the patient who is still living are all Rh+

Summary

An account is given of a similar and hitherto unknown clinical hematologic syndrome in two adult brothers with marked hemorrhagic diathesis diffuse pigmentation of the skin violent pain in the bones and panhemocytopenia In the younger brother there is also a certain degree of infantilism The elder brother died with all the symptoms of an intensive aplastic anemia in the younger brother the condition was stabilized after splenectomy The blood picture in both patients was characterized by a hyperchromic anemia with remarkable micro and macrocytosis and an increased number of reticulocytes In the younger brother increased fragility of the red blood cells and an elevated serum iron content were observed In both cases an unusual increase of the plasmocytic and reticular cells and of the tissue mast cells was noticed in the bone marrow and in the final stages of the dis-ase the marrow showed marked fibrosis

The disease is believed to be a variety in adults of the syndrome first described by Fanconi as a constitutional panmyelopathy occurring in children The illness is the result of a hereditary pathologic reaction of the reticulo histiocytic system and seems to have been caused by an anaphylactic allergic phenomenon The possibility is discussed that genetic connections may exist between this condition and other diseases such as certain osteoscleroses and Cooley s anemia which are characterized by simultaneous disturbances of the bone and bone marrow and by a similar blood morphology

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THE PATHOGENESIS OF ANEMIA IN ACUTE GLOMERULONEPHRITIS ESTIMATIONS OF BLOOD PRODUCTION AND BLOOD DESTRUCTION IN A CASE RECEIVING MASSIVE TRANSFUSIONS*

By CHARLES P EMERSON M D

ANEMIA is one of the familiar manifestations of Bright s disease and a frequent complication of uremia irrespective of the etiologic factors responsible for renal failure [1–3] A peculiarly intimate association exists between anemia and glomerulonephritis in relation to which this hematologic sign is of diagnostic and prognostic importance [4, 5] Conclusions regarding its pathogenesis are based on the experience of numerous investigators who have emphasized the consistent lack of signs indicating excessive blood loss or blood destruction and have succeeded in correlating the occurrence and severity of this anemia with the degree and duration of associated azotemia [2, 4, 6, 7] Hence the anemia associated with nitrogen retention is generally regarded as an example of erythropoietic failure Furthermore its refractoriness to erythropoietic stimulation with iron or liver therapy has been interpreted[8–11] as evidence that blood production in patients with renal decompensation is retarded in consequence of toxic inhibition of the bone marrow by retained nitrogenous metabolic products

This hypothesis although possibly correct as a premise mainly deduced through analogy by inference and by exclusion bears particular scrutiny little or no evidence of a positive and unequivocal sort having been marshalled in its support The toxic metabolite presumed to be implicated has thus far escaped identification and of all of the numerous chemical agents recognized as bone marrow depressants there is none known to exert comparable effects on the bone marrow or peripheral blood Finally it may be objected that the hematologic data cited from case reports in support of this concept including descriptions of reticulocytosis and alterations of bone marrow histology [5, 1–14] in some instances suggest an enhancement rather than a depression of erythropoietic activity in patients with nephritis and anemia

Transfusion studies designed to permit an estimation of the survival of injected donor red cells have contributed valuable information relative to the pathogenesis of various types of anemia particularly those associated with certain hemolytic syndromes [15, 16] This technic of investigation employing serial measurements of *the circulating blood volume in addition to selective agglutination counts* was applied in the study of a patient with an initial attack of early acute glomerulonephritis who presenting signs of moderate azotemia and progressive anemia was adjudged particularly suitable as a subject for detailed hematologic investigation Data were accordingly obtained which served as a basis for the relative evaluation of blood production blood loss and blood destruction as factors possibly implicated in the development of his anemia Appreciating the limited significance of the results obtained which pending confirmation from comparable investigations can

* The data utilized in this case report were obtained while on active duty with the Fifth (U S) General Hospital in the European Theater of Operations

hardly be evaluated in relation to other patients with Bright s disease the observa tions are nevertheless considered of sufficient interest to warrant description in the form of an individual case report

Case History and Initial Observations*

A 27 year old white American enlisted soldier was admitted to the hospital complaining of progressive swelling of the legs Two months before entry he had contracted an acute pharyngitis which completely subsided in the course of several days Thereafter he experienced persistent weakness and unusual fatigability Two weeks before admission he became aware of painless swelling of his lower extremities which increased and together with symptoms of general malaise headaches and anorexia occasioned his entry to the hospital

The physical findings on admission were those of a well-developed mal with pallid complexion and obvious pitting edema of the lower extremities His body temperature was normal arterial pre u 190/130 mm Hg height 168 cm weight 73 4 Kg (13 Kg in excess of his average weight prior to the present illness)

Initial laborato y data Urinalysis Specific gravity 1 015 albumin 4+ sediment (uncentrifuged) containing 15-20 r b c 2-4 w b c and numerous casts granular and cellular per high power field Blood hemoglobin concentration 13 1 Gm per cent red cell count 3 98 million per cu mm hematocrit reading 36 - leukocytes 8 200 per cu mm with normal differential count platelets 294 000 per cu mm Erythrocyte osmotic fragility normal sedimentation rat (Westergren) 17 mm in one hour Blood urea nitrogen concentration 25 mg per cent total serum protein concentration 4 4 Gm per cent Bl ed ing time (Duke) 3½ minutes clotting time (Lee White) 8½ minutes Stool examinations were negative for occult blood The initial throat culture contained beta hemolytic streptococci this organism failing to be demonstrated on re-examination after eight days

Methods of Study

The patient was observed for a period of fifty days during which he was essentially at complete bed rest maintained on a dietary regime restricted solely with respect to its sodium content Penicillin 120 000 units daily was administered intramuscularly from the fourth to the thirteenth day Otherwise apart from transfusions and albumin injections subsequently to be specified no therapeutic agents, hemopoietic diuretic or antibacterial were employed

Daily observations included measurements of the arterial pressure body weight fluid intake and urine volume Urinalyses were performed daily which included after the eighth day a quantitative (Esbach) estimation of the total urine albumin excretion Blood hemoglobin concentrations and icterus indices were determined with Klett photoelectric technics Blood urea nitrogen was measured colorimetrically after urease digestion and nesslerization and total protein concentrations by the procedure of Phillips and Van Slyke [17] Plasma volume determinations employing T 1824 dye were performed by a modification[18] of the method of Gibson and Evans [19] Calculations of the circulating red cell volume and total blood volume were based on the plasma volume and hematocrit values these computations involving a correction factor of -15 per cent applied to the calculated red cell volume to compensate for the relatively constant disparity between the large vessel hematocrit and the total body hematocrit [8]

Group-O donor blood freshly drawn into acidified glucose citrate anticoagulant

* Identifying initials of the patient have been deleted here and in the table nd figures at the r quest of the Technical Inform tion Office of the Surgeon General s Office

solution was employed in the first course of transfusions in preparation for the second series red cells from freshly obtained group-O blood were washed once and resuspended in 0 85 per cent saline solution At intervals following transfusions the concentration of donor cells was determined from these data it was possible to calculate the total volume of circulating donor cells and group-A recipient s cells Selective agglutination counts were performed by modifications of the Ashby technic utilizing dried anti A grouping serum a procedure that has been successfully applied in other investigations[21, 22] and has recently been evaluated by Young [3]

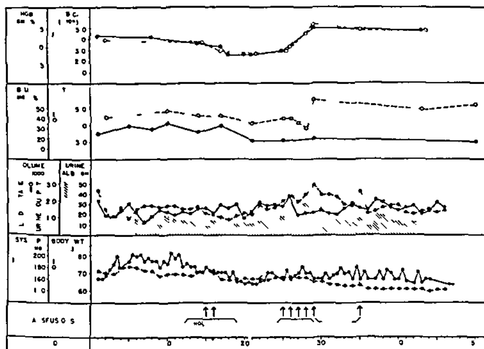


FIG 1 Hematologic blood chemical and clinical data on a case of acute glomerulonephritis receiving infusions of whole blood red cells and albumin

Course

The hematologic findings and metabolic data obtained in this case are charted in figure 1 which depicts the observed fluctuations in the hemoglobin concentration red cell count blood urea nitrogen total serum protein concentration fluid balance total albumin excretion body weight and systolic arterial pressure

It was evident on the fifteenth day that the patient s anemia which was of a normocytic normochromic type was progressing in severity the hemoglobin concentration having decreased from 13 1 to 11 2 Gm per cent and the hematocrit reading from 36 7 to 30 8 since admission to the hospital Blood volume measurements (table 1 and figure 2) indicated a total circulating red cell volume of 1080 cc representing a calculated deficit of approximately 900 cc or 45 per cent relative to

the expected volume for an average normal male of his stature The total blood volume was likewise deficient (approximately 20 per cent) this decrease being en

TABLE 1 —*Blood Studies in the Course of Transfusion Therapy*

Hosp Day	Hgb	Venous Hct	Donor Rbc (O)	Plasma volume	Total blood vol	Red cell volume Total	Group A	Group O	Retics	Icteric index	Blood urea N	Plasma proteins	Blood transfusions
	Gm %		%	cc	cc	cc	cc	cc	%		mg	Gm %	
1	13 1	36 7	0	—	—	—	—	—	—	—	25	4 4	
14	11 2	30 8	0	2880	3960	1080	1080	0	1 2	6	26	4 5	
15–16													Rbc (o) 470 cc Plasma 650 cc
17	10 0	28 5	41	3270	4370	1100	650	450	13 8	11	32	4 5	
21	8 6	23 8	37	—	—	—	—	—	8 9	—	17	4 1	
25	9 3	27 1	38	2770	3650	880	550	330	11 5	6	17	4 3	Rbc (O) 260 cc
26	—	33 1	48	—	—	—	—	—	—	6	—	4 4	Rbc (O) 245 cc
27	—	36 6	52	—	—	—	—	—	—	8	—	4 1	Rbc (O) 230 cc
28	—	41 6	57	—	—	—	—	—	—	9	—	3 8	Rbc (O) 240 cc
29	15 3	43 7	57	2450	4060	1610	690	9–0	–	14	20	5 5	
43	14 8	40 2	52	1880	4540	1660	800	860	—	6	—	4 9	
50	—	41 4	48	—	—	—	—	—	—	—	16	5 3	
Values expected in normal male ht 168 cm (20 24)				2850	4850	2000							

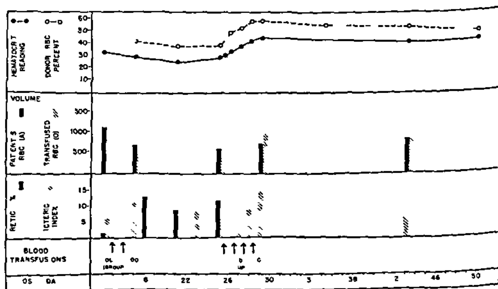


FIG 1 The influence of blood transfusions on anemia associated with acute glomerulonephritis

tirely accounted for on the basis of the decrease in red cell volume the plasma volume being within normal limits Blood loss via the gastrointestinal and renal

tracts had been insignificant hematuria continuing to be of microscopic degree and only an occasional stool containing a trace of occult blood during the entire period of observation

First transfusion series On the fifteenth and again on the sixteenth hospital day the patient received group-O whole blood totalling 470 cc of red cells and 650 cc of plasma A mild febrile reaction followed on each occasion and at the conclusion of the second injection he experienced transient severe lumbar aching pain The spleen promptly became enlarged and remained palpable for two weeks Hemoglobinemia and hemoglobinuria did not occur but there developed for the first time a mild transient icterus a relative decrease in urine output and an increase in albumin excretion

Blood volume studies and selective agglutination counts performed on the day following the second transfusion demonstrated that whereas the injected red cells had survived *in toto* a destruction of the patient s group-A cells had occurred this loss approximately equalling the volume of transfused erythrocytes (table 1 and fig 2) Thereafter during the nine day interval between the first and second series of transfusions the red cell volume continued to diminish the patient s cells decreasing by 100 cc (15 per cent) and the donor cells by 120 cc (27 per cent) This progression of anemia developed despite the onset of a persistent reticulocytosis which occurred abruptly following transfusion (fig 2)

Second transfusion series Between the twenty fifth and twenty ninth hospital days the patient was transfused with saline washed group-O cells derived from 2000 cc of whole blood all plasma having been removed The total red cell volume was thereby increased to 1610 cc or approximately 80 per cent of the expected normal value There was a recurrence of mild transient icterus which on the basis of selective agglutination counts was apparently due to the prompt destruction of those donor cells injected in the final transfusion No destruction of patient s cells resulted and those donor cells remaining at the conclusion of this series of transfusions survived thereafter in normal fashion less than 10 per cent being eliminated in the course of the ensuing two weeks A progressive increase in the patient s circulating red cells was demonstrable after the twenty fifth day their total volume being 250 cc or 45 per cent greater on the forty third day Although no subjective symptoms were associated with the cell transfusions a definite increase in proteinuria was noted immediately thereafter a phenomenon which had likewise followed the earlier transfusions of whole blood and the subsequent administration of crystalline human albumin

Discussion

This report concerns a patient hospitalized early in an initial attack of acute glomerulonephritis with manifestations of arterial hypertension hematuria albuminuria and nitrogen retention who was under continuous observation for fifty days During the first observation period when renal decompensation was maximal although by no means marked there developed a moderately severe normocytic normochromic anemia of a type commonly associated with nephritis Two weeks following the appearance of dependent edema the first clinical evidence of

his renal disease the venous hematocrit reading was 36 7 a reduction of approximately 20 per cent two weeks later the hematocrit was 30 8 approximately 30 per cent below normal but the calculated deficit in total circulating red cell volume at this time was 45 per cent Measurements of the plasma volume indicated that there had occurred no compensatory increase in the latter and that the true severity of the anemia judged solely on the basis of the red cell and hemoglobin concentrations had been obscured as a result of a reduction in the total blood volume Alterations of a similar character in patients with acute nephritis have been reported by Harris and Gibson [5]

Rapid red cell depletion of the degree exhibited by this patient is difficult to explain solely on the basis of erythropoietic depression due to toxic inhibition of the bone marrow to a metabolic defect or to a nutritional deficiency Even assuming a complete cessation of blood production the decline in the red cell volume occurred *at approximately twice the expected rate* [8] *of 0 8 to 1 0 per cent per day* unless this bone marrow aplasia is considered to have occurred at the time of the antecedent pharyngitis The latter possibility can hardly be discarded but such an hypothesis presumes that the initial observations of hematocrit and hemoglobin concentration were misleading the plasma volume at that time being considerably lower than when first determined two weeks later a supposition for which there is no basis Complete bone marrow inactivity is in any case an improbable explanation for the observed anemia on the grounds that the reticulocytes although not numerous (1 2 per cent) before transfusion therapy were nevertheless present It is of interest that the presence of reticulocytes in the peripheral blood has consistently been described in case reports published in relation to this problem whatever interpretations may have been adduced from the hematologic data obtained

Significant blood loss having been adequately excluded during the entire period of observation it is inferred that excessive and uncompensated blood destruction must have been responsible for the rapid development of anemia in this case Observations following the first series of transfusions tended to confirm this evaluation of the mechanisms involved The patient blood group A received 1000 cc of group O whole blood in the course of twenty four hours a procedure which precipitated a mild hemolytic crisis with prompt destruction of 430 cc of his own cells Inasmuch as the donor erythrocytes quantitatively replaced the destroyed recipient cells there occurred no significant change in the severity of the anemia An important contributing factor in this response was unquestionably the presence of incompatible isoagglutinins in the injected donor blood the hemolytic effect of which has been previously described Unfortunately the titer of anti A isoagglutinins in the injected material was not determined It may be stated however that no instance has been observed [1] in which a comparable degree of hemolysis was produced by the first transfusion of plasma or whole blood containing incompatible isoagglutinins in very high titer hence this patient must have been unusually susceptible to the hemolytic effect of the universal donor blood he received Of far greater significance are the observations pertaining to the subsequent fate of the normal donor erythrocytes which were eliminated at an average rate of 3 per cent per day or more than three times the expected rate Depletion of the patient s own

cell population also continued to be excessive (1 6 per cent per day) but their net loss occurred less rapidly which may be explained on the basis of a sudden increase in blood production evidenced by a concomitant reticulocytosis (fig 2)

This sudden and unexpected increase in reticulocytes indicating enhanced erythropoietic activity immediately following transfusion deserves particular mention Immediately preceding this therapy the reticulocyte count was 1 2 per cent immediately thereafter the percentage had increased to 13 8 The precise explanation for this phenomenon is not evident but it is of interest that the peak reticulocytosis occurred prior to a further substantial reduction in the venous hematocrit or hemoglobin concentration hence the stimulus for increased bone marrow activity was was not primarily an increase in bone marrow hypoxia Moreover inasmuch as it occurred at a time when the elevation of blood urea nitrogen was almost maximal one is tempted to reject the hypothesis that the previous inadequacy of blood production was due to toxic inhibition of the bone marrow as a result of nitrogen retention or to other unexcreted metabolites It is possible that the resumption of normal erythropoietic activity *displayed at this time* was *related to the increased* hemolysis provoked by the administration of incompatible isoagglutinins that it occurred not as a result of increased anemia donor erythrocytes having been substituted almost quantitatively for the patient s hemolyzed red cells but due to the stimulus of some hemopoietically effective material derived from the latter Or the donor blood may have been the source of an erythropoietic agent of which there had been a previous deficiency Whatever the true explanation blood formation proceeded thenceforth at an increased rate although temporarily outpaced by blood destruction

As a result of the second series of transfusions involving the administration of plasma free red cell suspensions the patient s anemia was practically relieved In the course of four days the hemoglobin concentration was increased from 9 3 to 15 3 Gm per cent and the hematocrit reading from 27 1 to 43 7 the total red cell volume was almost doubled A significant proportion of the injected cells were hemolyzed in the process of their preparation or were eliminated very promptly following the injection Nevertheless the subsequent fate of this donor blood which survived normally together with data indicating a progressive increase in the patient s red cell population suggest that abnormal blood destruction had ceased and that blood formation was occurring at a normal rate The factors responsible for this reversion to a normal hematologic status can not be positively identified on the basis of the available evidence It is of interest however in view of the well known correlation between the anemia of renal disease and the degree of nitrogen retention that during the first twenty hospital days when signs of increased blood destruction and impaired erythrogenesis were most prominent the blood urea nitrogen concentration ranged from 25 to 34 mg per cent (average 30 mg per cent) whereas during the subsequent thirty days when erythropoiesis and hemolysis were normal the blood urea nitrogen did not exceed 20 mg per cent (average value 17 5 mg per cent) No relationship was observed between the hematologic status and the grade of hematuria and proteinuria or fluctuations in the total circulating protein

A final comment is warranted regarding the influence of transfusion therapy on other manifestations of nephritis in this case No evidence can be adduced that the course of the arterial hypertension, which was one of gradual improvement was in any way affected by these maneuvers Hematuria and albuminuria persisted without remission throughout the period of study The transient elevations of total urinary albumin excretion following each series of transfusions whether involving the injection of whole blood washed red cells or purified albumin, presumably reflect an increased renal blood flow and glomerular filtration attending this therapy Similar increases in proteinuria following the administration of albumin in cases of nephritis have been described by Thorn et al [7] The progressive improvement in renal function as measured by changes in body weight indicating increasingly effective water and sodium clearance, is readily attributable to the natural course of this patient s disease rather than to variations in the degree of anemia Thus there was less water retention on the twenty fifth hospital day, when his body weight was 69 kilograms his blood volume 3650 cc and hematocrit 27 1 than on the fourteenth day when his body weight was 73 kilograms blood volume 3960 cc and hematocrit 30 8 Similar conclusions obtain with respect to the observed reduction between the eighteenth and twenty first days in the blood urea nitrogen concentration these data being obtained in a patient whose renal decompensation was never severe and whose clinical course was entirely consistent with one of progressive spontaneous improvement

Summary and Conclusions

1 A 27 year old patient with an initial episode of acute glomerulonephritis was observed over a fifty day period studies being directed primarily in an attempt to define the mechanisms responsible for a rapidly developing anemia Hematologic data including serial blood volume measurements and selective agglutination counts were obtained before and after the introduction of massive transfusion therapy

2 The administration of group O whole blood containing incompatible anti A isoagglutinins in the first series of transfusions failed to improve the anemia but initiated a sustained reticulocyte response Following this therapy there was evidence of increased blood destruction involving both the recipient s and the normal donor erythrocytes

3 Data obtained following a second series of transfusions employing plasma free group-O red cells administered during a recovery phase when renal function had improved indicated that blood destruction had largely abated and that hemopoietic activity was normal

4 Two factors of undetermined origin are believed to have been implicated in the pathogenesis of anemia in this case one the occurrence of abnormally rapid blood destruction and the other impairment of blood formation Both phenomena were associated with the presence of nitrogen retention despite which however a prompt erythropoietic response followed the transfusion of whole blood with quantitative replacement of patient s red cells with donor erythrocytes suggesting that previous bone marrow inactivity was not attributable to toxic suppression

ACKNOWLEDGMENT

The author is greatly indebted to Dr Richard V Ebert formerly Major M C A U S for his valuable assistance in the planning and conduct of this experimental case study

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PROPHYLAXIS OF HOOKWORM ANEMIA DEFICIENCY DISEASE

By W O CRUZ M D AND R PIMENTA DE MELLO M D

IN VIEW of present knowledge of hookworm anemia it has become evident that qualitatively and in conjunction with helminthic infestation deficient nutrition is of importance in the genesis of this disease The possibility of curing the anemia even though the intestinal parasitism remains has provided us with the opportunity of observing which symptoms and clinical signs result from a hemoglobin deficiency and which are caused directly by the presence of the helminths Contrary to what might be expected with the exception of intestinal hemorrhages and eosinophilia all other pathologic changes disappeared as the blood became normal So great is the importance of these symptoms and signs that yield with the treatment by iron and so insignificant are those that remain that we should in this case consider the anemia not as a syndrome connected with the signs but as the disease itself The specificity of the treatment of anemia by iron and the astonishing nature of the cure are the usual characteristics of conditions of deficiency

Up to the present time prophylaxis of hookworm anemia has been considered as the prophylaxis of a disease which is strictly parasitic in origin The methods are difficult and costly amounting almost to radical changes in the firmly established habits of a population (use of shoes) or sanitary engineering measures amounting almost to sudden civilization of backward zones (construction of privies etc) These classic methods of prophylaxis consisting in avoiding the infestation of man by *Ancylostoma* have been of no practical effect with respect to the incidence of the anemia

If we consider the prophylaxis from the point of view of the second agent in the etiologic complex of hookworm anemia i e qualitative nutritional deficiency a different plan of prophylactic campaign can be outlined The application of iron in prophylaxis is not sufficient to eliminate completely the disease from a community and in addition it requires periodic application On the other hand this method is one of the easiest to apply when it is duly supported by the proper public health laws

Following these principles Cruz and de Mello[1] attempted to create the bases for a prophylaxis of hookworm anemia considered as a deficiency disease (similar to the prophylaxis of endemic goiter) This consisted in adding an iron salt hematologically active to the foods habitually eaten by the lower social classes The difficulties encountered were considerable as compared with the prophylaxis of endemic goiter In the latter 0 005 Gm of potassium iodide are sufficient whereas in hookworm anemia we had to use a much higher dose of usable iron salt Various trials were made not only for choice of food but also of the iron salt with highest therapeutic value and stability The authors concluded that the mixtures of ferrous sulfate with manioc flour and of ammoniacal ferric citrate with bean gravy were

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Aided by a grant f om Dr Guilherme Guinle

[5] HARRIS A W AND GIBSON J G 2nd Clinical studies of the blood volume VII Changes in blood volume in Bright s disease with or without edema renal insufficiency or congestive heart failure and in hypertension J Clin Investigation *18* 527–536 1939
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relation to the basic metal for the respiratory function This metal is a vital raw material for reconstitution of respiratory pigment and the organism is entirely dependent on the reserves supplied to it by nutrition to maintain a normal hemo-

TABLE 2

Iron Compound	Foodstuff mixed	Color of mixture	Taste	Hematologic action at therapeutic dose
Iron carbonat	Kitch n salt	brown	o	–
	Flour	brov n	o	–
	Sugar	brown	o	–
Iron glycerophosphat	Kitchen salt	y llowi h	+	+
	Kitch n salt	o	+	+
	Sugar	y llov ish	+	+
Iron proto-oxalat	Kitchen salt	y llowish	o	+
	Flour	y llo ish	o	+
	Sugar	y llowish	+	+
Iron pyrophosphate	Kitchen salt	hit	o	+
	Flour	o	+	+
	Sugar	o	+	+
Ammoniacal iron sulfat	Kitch n salt	dark yellow	+++	++
	Flour	o	+++	++
	Sugar	darkish	+++	++
	Sugar	darkish	++	++
Iron phospha e	Kitchen salt	dark green	+	++
	Flour	grey	+	++
	Sugar	greenish grey	+	++
Iron albuminate	Kitchen salt	brov n	o	–
	Flour	*brown*	o	–
	Sugar	brown	o	–
Ammoniacal ferric citrate	Kitchen salt	y llow	+++	++
	Flour	o	+++	++
Tartrate of iron and potassium	Kitchen salt	light brown	++	–
	Kitchen salt	light brown	++	–
	Flour	brown	o	–
	S gar	dark brown	o	–
Iron benzoate	Kitchen salt	d rk brown	+++	–
	Flour	dark bro n	+++	–
	Sugar	dark brown	+++	–
Iron lactate	Kitchen salt	greenish	+++	+
	Flour	o	++	+
	Sugar	brown	++	+
Ferrous sulfate	Kitchen salt	yellow	++	++++
	Flour	o	o	++++
	Sugar	yellow	++	–

globin metabolism *Accordingly when the helminths withdraw blood from the* body they withdraw essentially the iron metal Therefore each helminth represents a unit of consumption in the iron balance in the body This unit will increase in importance in proportion to the decrease of iron in the circulation of the host *It is known* that in mammals the total amount of blood is approximately 10 per

sufficient not only to cure but later to prevent the fall of blood values during long periods of time in individuals who were heavily infested Various cases were described,[1] a summary of which may be seen in table 1

With the various experimental mixtures used it was attempted to fulfill the following requirements taste stability appearance hematologic efficiency and low cost Only two were found to be satisfactory ferrous sulfate mixed with manioc flour and ferric ammonium citrate brown added to bean gravy The salts

TABLE 1

Name	Age	Weight	No of uo m el ms nat d	No f u rms pe k l b dy u g l	Intensity of infestation	H m glob let l ma n ta ed (Gm 100 ml blo d)	Days ma ntai ed	I on s lt used	Daily dose	Equ le t metal c i	F ed t which added
	y s	k l s							Gm	Gm	
Pedro	8	23	500	22	VI	10 00	40	Ferrous sulfate	0 50	0 185	Manioc flour
Pedro	8	23	500	22	VI	9 50	85		0 25	0 092	
Argentina	0	45	717	16	VI	10 25	82		0 50	0 185	
Argentina	20	45	717	16	VI	10 25	91		0 25	0 092	
Carlos	45	50	758	15	VI	9 00	80		0 50	0 185	
Edno	13	26	350	13	V	11 00	82		0 50	0 185	
Edno	13	26	350	13	V	11 50	91		0 10	0 037	
Jose V	16	38	499	13	V	11 25	11		0 50	0 185	
Jose V	16	38	499	13	V	11 00	45		0 25	0 09	
J Magalhae	9	28	300	11	IV	10 25	90	Ammoniacal ferric citrat	1 00	0 10	Bean gravy
Valdir	12	30	280	9	IV	9 50	87	Ferrous sulfate	0 50	0 185	Manioc flour
Mario	18	46	230	5	IV	10 00	65		0 50	0 185	
Mario	18	46	230	5	IV	10 00	128		0 25	0 092	
Delvair	9	26	130	5	IV	10 50	8		0 50	0 185	
Maria	19	45	180	4	III	11 00	80		0 50	0 185	
Maria	19	45	180	4	III	11 75	90		0 10	0 037	

See table 3

listed in table 2 were also tried but did not fulfill the requirements and their use was not continued

With regard to the infestation index listed in table 1 we should keep in mind that according to present knowledge the contribution of the helminths to th formation of anemia appears to be exclusively through their blood sucking activi ties The hemorrhages caused by this action have a distinctive significance in the physiology of the blood The organism reacts in various ways according to the hematic constituents lost in a hemorrhage It seems to possess an unlimited quan tity of protein for reconstitution of the red blood cell strome of globin and of amino radicals present in the chemical structure of heme This is not the case with

Digestive system Epigastric region is sensitive to touch but does not present spontaneous pain No constipation in last two months attacks of diarrhea have been frequent. Liver and spleen not increased in volume

Circulatory system Pulse light soft and rhythmic with 84 pulse beats per minute Blood pressure is 110/75 Lack of thrill in neck vessels Ictus weak located in fifth intercostal space one centimeter inside the hemiclavicular line Systolic murmur (+–+) soft audible at point and at base Not spread by any forces Diminishes in intensity at beginning of inspiration and on the other hand increases when the individual lies down or when the auscultation point is pressed with stethoscope In the presystole the auricular sound is heard in the mesocardiac region A2 and P2 are equal and normal

Respiratory system and other systems normal

Sojourn in hospital The stay of the patient in the hospital was not abnormal due to two factors not connected with the Ancylostomiasis (1) a secondary infection in some lesions of the scabies mentioned (2) a dental abscess both occurring when health conditions were very poor With the use of iron the symptoms and signs caused by the anemia diminished immediately At the end of the first week the

Table 4—Hematologic Tests

	Date												
	1945										1946		
	1 15	1 –	2 4	2 15	–3	4 19		8 1	9 –	11 19	1 3	2 6	4 1
	Days of treatment												
	0		16	–	3–	91	1–3	2–4	[illegible]	3–1	3–5	3–8	444
Red blood cells (10⁶/ml)	1 –	1 9	– 6	3 7	3 –	4 9	5 1	4 5	4 5	4 6	4 3	3 8	5 0
Hemoglobin (Gm /100 ml blood)	1 5	4 4	6 –	8 8	8 –	11 –	1– 0	9 6	11 2	9 0	1 2	8 6	11 6
Hematocrit (%)	1–	14	1	–9	30	35	38	34	36	33	3–	9	38
Mean corpuscular volume (cubic micra)	58	–3	80	–8	80	–1	–5	–6	–5	–2	–5	–6	–6
Mean corpuscular hemoglobin (micro-micrograms)	1	3	–4	–4	–	3	–3	1	–3	20	–4	3	3
Mean corpuscular hemoglobin concentration (%)	21	31	9	30	–	3–	3–	–8	31	––	3–	30	31

malleolar edema no longer existed Urine examinations made immediately after the patient was admitted to the hospital and subsequent examinations showed nothing to indicate that renal function was affected Appetite was always good The attacks of diarrhea disappeared Forty days following beginning of treatment the patient had gained 6 kilos weight Color of skin and of mucosas practically normal for our environment at end of February that is 45 days after admittance Tonicity had regained tonus Physical resistance permitted the practice of active exercise without reappearance of dyspnea and palpitations Heart beat remained about 70 per minute Blood pressure not changed systolic continuing between 105 and 115 and diastolic at 75 mm Hg Beginning the middle of March systolic murmur no longer heard only first sound found to be extended at point Auricular sound heard only when the heart because of the requirements of physical effort became hyperactive No opportunity to make radiologic study of this case

We accompanied clinical course of the anemia with frequent electrocardiograms We will analyze only two spaced about three months apart The others are transitional between these two or repeat the second which represents so to speak the final modification observed

In figure 1 (January 16 1946) and figure 2 (April 9 1946) the second (fig 2) shows the following modifications when compared with the first

cent of the body weight Hence the damage caused by a worm will be less important in an adult of 60 kilos than in a child of 20 kilos This means that the intensity of infestation can be expressed only by a relationship between the number of worms living on the intestine and the mass of circulating blood or roughly the body weight of the host Based on these data we suggest that the intensity of infestation from *Ancylostoma* be figured according to table 3

In order to determine approximately the number of helminths per kilo of body weight based on egg counts the following formula is used $\frac{N}{18P}$ in which N represents the number of eggs per gram of feces and P the weight of the individual expressed in kilograms Usually the infestation occurs with an equal number of male and female helminths and as the females of the Necator are responsible for eliminating 36 eggs per gram of stools we should divide the egg count by half of 36, which explains the factor 18 in the denominator of the formula Therefore for

TABLE 3

Intensity of infestation—Groups	Helminths per kilo of body weight
I	0
II	0– 0 9
III	1– 4 9
IV	5– 9 9
V	10–14 9
VI	over 15

example in a child 31 kilo body weight with 5 000 eggs per gram of feces we have $\frac{5000}{560}$ = 8 9 helminths per kilo of body weight a case belonging to group IV of our classification

Following these studies on the administration of iron in the prophylaxis of hookworm anemia considered as a deficiency disease, it would doubtless be very important to determine the minimum dose of salt to be used in order to maintain the blood values at a normal level For this purpose we submitted a patient with a high index of infestation to several doses of ferrous sulfate added to the food

CASE REPORT

C G 22 years old raidroad worker white Brazilian resident of Mage Weight 45 kilos Admitted to the hospital on January 11 1946 Discharged April 4 1947

Patient complains of extreme weakness is easily tired has dyspnea and palpitation after making the slightest physical effort Can not say for certain when illness commenced the symptoms appeared and progressed in unnoticeable manner Says he had no venereal or rheumatic past Although living in malaria zone informs never had malaria Drinks alcohol in moderation

General examination Asthenic badly nourished individual Skin yellowed visible mucosas highly discolored almost white Lesions of chronic scabies spread over trunk abdomen base of thigh and hands In the malar region on both sides and as far as the edges of the nose symmetrical irregular zones of dark coloring and a little shiny can be noted On malleoli slight edema less than one month old No decrease or changes in appetite Teeth are in poor condition Tongue is white broadened and marks of teeth can be seen on tip

Digestive system Epigastric region is sensitive to touch but does not present spontaneous pain No con tipation in last t o months attacks of diarrhea have been frequent Liver and spleen not increased in vol me

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	1946										1947		
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	D y f ob rvat												
	0	7	16	2	37	91	1 3	204	219	301	345	3 8	444
Red blood cells (10 /ml)	1 2	1 9	2 6	3 7	3 7	4 9	5 1	4 5	4 8	4 6	4 3	3 8	5 0
Hemoglobin (Gm /100 ml blood)	1 5	4 4	6 2	8 8	8 2	11 2	12 0	9 6	11 2	9 0	10 2	8 6	11 6
Hematocrit (%)	7	14	21	29	30	35	38	34	36	33	32	29	38
Mean corpuscular volume (cubic micra)	58	73	80	8	80	71	75	76	75	72	75	6	76
Mean corpuscular hemoglobin (micro micrograms)	12	23	24	24	22	23	23	21	23	20	24	23	23
Mean corpuscular hemoglobin concentration (%)	21	31	29	30	27	32	32	28	31	27	32	30	31

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In figure 1 (January 16 1946) and figure 2 (April 9 1946) the second (fig 2) shows the following modifications when compared with the first

1 Slight rotation of electric axis of the QRS to the left
2 Increased voltage on wave length T in D1 and in precordial positions left of the ictus
3 *Positivity of wave length T in V3*

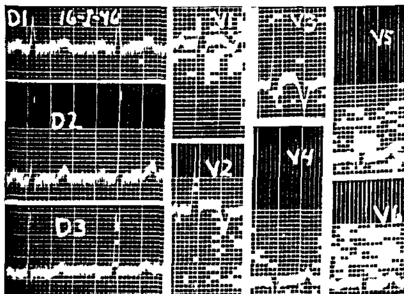


Fig 1

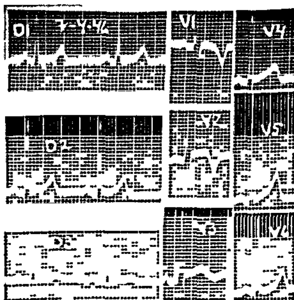


Fig 2

The electrocardiographic changes may partly be due to changes in the position of the heart Increase of wave length T in precordial positions left of the ictus in

D1 is probably due to changes of the process of repolarization of ventricular myo cardium caused by better nutritive conditions of muscular fibers

We started the therapy with iron administering ferrous sulfate 1 0 gram daily mixed with manioc flour a food widely used in certain regions of Brazil The blood values increased rapidly from 2 0 grams to 7 0 grams of hemoglobin per 100 cc of blood We decreased the dose to 0 5 Gm daily always added to the same food At the end of two months the hemoglobin value was practically normal (11 0 grams per 100 cc of blood) We then tried to determine the minimum dose necessary to maintain a relatively normal hemoglobin level The administration of 0 1 Gm daily was insufficient to maintain this level and hemoglobin decreased from 11 0 Gm to 8 0 Gm at the end of 110 days Experiments with 0 2 Gm of ferrous sulfate however proved to be a sufficient dose to avoid the decrease and enough

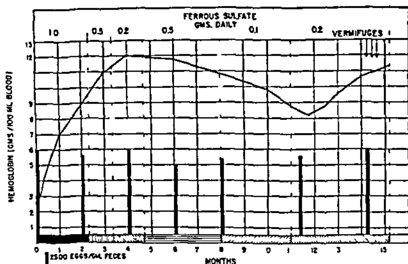


Fig 3

to keep the blood levels normal for 100 days A graphic presentation of this observa tion is given in figure 3

Attention should be called to the extreme clinical changes which occurred in this patient after iron was administered When he was hospitalized the patient was entirely apathetic without strength to move remaining in bed for a considerable portion of the day As the blood values became normal his functional activity was restored In the final period of treatment he remained semi hospitalized and worked in our laboratory In carrying out his work he walked daily about two kilometers from the hospital to the place of work He became quite active as can be seen by the fact that several times a day he went to the animal house about 200 meters away going up a steep incline and climbing three flights of stairs on re turning to the laboratory He no longer felt the symptoms of which he complained when he was hospitalized He became from all points of view a perfectly normal individual

Eggs of Necator were counted periodically, for control of the biologic activity of the helminth and the persistence of the degree of infestation

At the end of the trial period, five vermifuges (carbon tetrachloride 1 8 ml + Chenopodium oil 0 6 ml) were administered at weekly intervals Helminths to the number of 1051 were eliminated representing one of the most heavily infested cases we have observed (infestation index = 24 helminths per kilo of body weight) The fact should be kept in mind that the number of helminths eliminated represents a minimum since it is easy to understand that not only do some escape at time of counting but also others disappeared by natural death during the period of hospitalization

Summary

1 In individuals severely infested with *Ancylostoma* or *Necator* it is possible to maintain the normality of blood value by the administration of a sufficient dose of an iron salt

2 The minimum dose necessary to maintain normality of the blood in an individual weighing 45 kilograms with 1051 helminths was 0 2 Gm daily of ferrous sulfate administered in mixture with manioc flour

3 The patient observed became clinically normal two weeks after the beginning of blood regeneration up to the end of the trial period one year later In this period with the various doses of iron tried hemoglobin varied from 8 0 to 11 0 per 100 ml of blood

ACKNOWLEDGMENT

We owe thanks to the kindness of our colleague Dr Genard Nobrega for the case report and electrocardiographic study of the patient

REFERENCE

1 Cruz W O and Pimenta de Mello R Prophylaxis of hookworm anemia-deficiency syndrome Mem Inst Osw Cruz 42 401–448 1946

IRREVERSIBLE TOXIC INCLUSION BODY ANEMIA

A Rarely Recognized Syndrome Clinical and Experimental Studies

By M H Fertman M D and Charles A Doan M D

A PATHOLOGIC entity apparently rarely recognized and unreported in recent American and British hematologic clinical literature is a form of refractory anemia characterized by peculiar inclusion bodies in the circulating red blood cells First reported by Heinz in 1890[1] and noted by Ehrlich in 189_ in experimental poisonings with pyrodine dinitrobenzol and other related compounds Heinz Inner Korpern may be readily identified in plain dried blood films and are described in wet preparations stained with brilliant cresyl blue or Nile blue sulphate as deep blue eccentrically placed spheres of varying size and number within the erythrocytes[3] They are readily distinguished from Howell Jolly bodies siderocytosis and classic reticulocytosis by their characteristic appearance distribution and staining reactions[4] They have gone undetected in most routine clinical laboratories probably because in the usual Wright Giemsa stained and mounted blood films prepared for microscopic study they are difficult to demonstrate

Our own attention has been currently focused upon this phenomenon by the discovery of its occurrence in the blood of an elderly physician with an unexplained refractory anemia which terminated fatally despite the use of all available therapeutic measures [9]

Clinical Observations

Dr M #4410193 a 71 year old white male physician was admitted to the Hematology Service University Hospital with complaints of weakness anorexia and nausea of four weeks duration Attacks of angina pectoris of increasing severity and frequency had been noted for approximately four years Eight weeks prior to this admission the patient first began to experi nce some dyspnea orthopnea and moderate pedal edema

Examination re eal d an obese elderly male showing marked pallor of the skin and mucous membranes with a sl ght icteric pigmentation His tongue showed no atrophic changes and lymphadenopathy was nowhere apparent The chest wa incre sed in the antero-posterior diameter and fine scattered rales were heard in both lung bases The heart was moderately enlarged to the left on percussion A soft blowing apical systolic murmur was present The blood pressure was within normal limits The liver edge was palpable 6 cm below the right costal angle the spleen was not enlarged Rectal examination revealed a diffusely enlarged firm prostate with no tenderness and no nodules There was slight pitting edema of the ankles Vibratory and position sense and deep tendon reflexes were physiologic

The patient was temper ture free th oughout his entire course in the hospital except for one trans tory mild thermal post transfusion reaction

Electrocardiograms demonstrated sinus tachycardia and low voltage with evidence of myocardial damage X ray examination of the chest sho ed moderate enlargement of the heart with diffuse densities in both lung fields suggestive of some cardiac congestion Roentgenographic examination of the stom ch and lower gastrointestinal tract showed no evidence of organic pathology X ray plates of the skull long bones and bony pelvis were entirely normal

From the D ision of Medical Research Department of Med cine Ohio State University Col mb s Oh o

Eggs of Necator were counted periodically for control of the biologic activity of the helminth and the persistence of the degree of infestation

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[1] Cruz W O and Pimenta de Mello R Prophylaxis of hookworm anemia-deficiency syndrome Mem Inst Osw Cruz 42 401–448 1946

The bone marrow on repeated examinations appeared grossly and microscopically to be markedly hyperplastic The erythroid elements were chiefly responsible for the cellular hyperplasia with a left shift to early erythroblasts and megaloblasts There was also the hypochromia of iron deficiency The myeloid elements showed a moderate left shift with more than the usual number of qualitatively normal myelocytes B but no myeloblasts There was an increase in small normal young megakaryocytes No foreign tumor cell invasion was seen There were scattered phagocytic clasmatocytes Inclusion bodies were observed rarely in the immature erythroid elements in our specimens of bone marrow

On examination of the patient s blood with brilliant cresyl blue there were found in addition to the classic reticulocytes certain inclusion-containing mature red cells With this stain the inclusions appeared as blue green globules of irregular shape varying in size from barely perceptible dots to spheres almost 2 micra in diameter They occurred sometimes singly sometimes in great numbers within the cell They were most often concentrated at or near the cell membrane and on occasion were seen in various stages of extrusion from the cell They were found to be lysis stabile and resistant

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The staining properties and morphology of these particular inclusion bodies distinguish them from Howell Jolly bodies and siderocyte inclusions [5] Howell Jolly bodies are readily stained in Wright and Giemsa fixed preparations and appear to be dark reddish blue dots The siderocytes are characteristically identified with the Prussian blue reaction which leaves a heavy deep blue iron precipitate within the red blood cell

Experimental Observations

Following the discovery of inclusion bodies in the patient s erythrocytes it was determined that erythrol tetranitrate was the only drug which had been taken for many months prior to and coincident with the development of the anemia and to which the patient may have developed an idiosyncrasy

Since this peculiar anemia persisted and continued to progress even several weeks after the presumed toxic agent was discontinued some permanent irreversible damage must have been suffered by the erythropoietic tissues Experimental procedures were therefore undertaken in an attempt to further identify the etiologic agent and establish the mechanism involved in this fatal anemia

EXPERIMENT I EFFECT OF THE PATIENT S PLASMA UPON NORMAL RED CELLS IN VITRO

Preliminary in vitro studies were conducted to determine whether there were any toxic substances present in the fresh whole plasma of the patient which would

The admission blood study revealed 2 331 000 red cells per cu mm 5 6 grams of hemoglobin per 100 cc and 16 100 white cells with 83 per cent mature motile neutrophils and 13 per cent normal, small lymphocytes Serial urinalyses and kidney function tests were consistently normal The sedimentation rate blood phosphorus and phosphatase and blood urea nitrogen were within normal limits The t s for Bence Jones protein in urine and plasma were repeatedly negative The hippuric acid excretion was only 1 88 Gm prothrombin time 58 8 per cent and blood proteins 4 68 mg with 2 83 mg albumin and 1 96 mg globulin Gastric analysis revealed a normal amount of free hydrochloric acid and stool examinations showed no occult blood

During his fifty nine days of observation in the hospital the patient was given transfusions of whole blood and washed resuspended red blood cells totaling 6 liters His red cell count ranged between 1 810 000 and 3 980 000 per cu mm Because of the macrocytic anemia with a megaloblastic bone marrow he was given a therapeutic trial of a concentrated form of liver extract reticulogen 20 units daily for seven days This was later repeated for a ten day period Ferrous gluconate 5 grains three times daily was administered for fifteen days in an effort to correct the hypochromia The patient was digitalized and received aminophyllin for his cardiac status Other supportive treatment included high vitamin B complex supplement

The reticulocyte count which was 14 8 per cent at the time liver extract was first instituted rose to a peak of 22 8 per cent after seven days but was not followed by any significant increase in the total circulating red blood cells Irrespective of therapy the reticulocytes varied between 3 per cent and 15 per cent throughout the clinical course of the anemia

On the twenty third hospital day the mature red blood cells stained with brilliant cresyl blue were first observed to contain atypical inclusions distinguishable from the regular reticulum As many as 13 4 per cent of the erythrocytes contained these bodies Inclusion bodies were noted from then on consistently in all daily preparations in numbers varying from 1 to 16 per cent How long these inclusions may have been present in the patient s circulating red cells prior to their detection is a matter of conjecture

The patient was resurveyed with reference to a possible toxic etiology for his refractory inclusion body anemia Attention was focused on the 500 ½ grain erythrol tetranitrate tablets taken orally over the preceding year for his angina pectoris Except for the rare use of nitroglycerine in cardiac crises no other drug had been taken There was no history of food idiosyncrasies or other allergic sensitivity The patient a physician stated emphatically that he had never been seriously ill or anemic in a long healthy life until the present illness

The patient was discharged at his own request on the fifty ninth hospital day At this time his peripheral blood showed 3 160 000 red cells per cu mm with 0 6 per cent reticulocytes 7 1 grams of hemoglobin and 16 2 per cent inclusion body erythrocytes His white count had fallen to 3100 per cu mm with 44 per cent neutrophils 8 per cent eosinophils and 48 per cent normal lymphocytes His platelet count was always adequate

The anginal attacks continued with frequency and severity despite bed rest digitalis and aminophyllin Pallor weakness and physical debility were progressive The red blood cells continued to show marked macrocytic hypochromia polychromatophilia anisocytosis poikilocytosis and the presence of inclusion bodies Fatal termination occurred one month after discharge from the hospital Post mortem examination was refused

Cytologic Studies of the Peripheral Blood and Bone Marrow

The peripheral blood showed extreme bizarre poikilocytosis and anisocytosis with large individual macrocytes and hypochromia The reticulocytes were not unusual in appearance as many as 14 8 per cent being found in the peripheral blood prior to liver therapy No antianemic hematopoietic liver iron vitamins B and C had any significant effect either on erythropoiesis or the hypochromia Platelets occurred singly and in large clumps and occasional individual thrombocytes showed some qualitative changes which included anisocytosis with sparse granulations (giant platelets) No pathognomonic alterations in the white blood cells were observed

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affect normal human red blood cells Venous blood was obtained from a normal subject whose blood grouping and Rh type were the same as the patient s These normal cells were separated and resuspended in the plasma obtained from the patient and kept in a refrigerator at 10 C Daily examination with brilliant cresyl blue over a five day period failed to reveal the development of any inclusion bodies in the borrowed red blood cells

Animal experimentation was then invoked utilizing two fundamental approaches (1) transfer of laked blood and fresh plasma was made by infusion from the patient into rabbits (2) both rabbits and cats were subjected to erythrol tetranitrate and allied compounds and the red blood cells studied for inclusion bodies

EXPERIMENT 2 EFFECT OF THE PATIENT S PLASMA AND LAKED RED CELLS UPON THE RABBIT

Since the patient s red blood cells contained the abnormal inclusion bodies it was felt that a toxic factor might be found either in the patient s plasma or in his red cells

Inclusion bodies did not appear in the erythrocytes of the rabbits which received intravenous injections either of plasma or of laked red blood cells from the patient R 1A and R 2A each received one 10 cc injection of plasma and the blood was followed for four days R 1 and R 2 received two injections of plasma (5 and 30 cc and 10 and 20 cc respectively) on consecutive days and died within thirty to forty five minutes after the second dose R 3 and R 4 received three intravenous injections of laked blood (5 10 and 5 cc and 1 cc 15 cc , and 5 cc respectively) R4 died immediately following its third injection No significant postmortem changes were noted in the spleen bone marrow or other organs of the three rabbits which died

EXPERIMENT 3 EFFECT OF SODIUM NITRATE AND ERYTHROL TETRANITRATE UPON ERYTHROPOIESIS IN THE RABBIT

Two rabbits (R 1A R 4A) were then given massive doses of sodium nitrate every day subcutaneously Necrosis at the site of injection necessitated substitution of the oral route via the stomach tube on the fifth day In R 4A the dose was doubled from 100 to 200 mg /Kg on the fifth day and death occurred on the seventh In R 1A the dose was increased from 500 to 1000 mg /Kg on the fifth day and to 2000 mg /Kg on the tenth Death occurred on the eleventh day

R 3 and R 2A received 50 to 300 mg /Kg doses of erythrol tetranitrate via stomach tube daily over a period of more than two weeks R 3 was followed for three days after completion of a sixteen day course of the drug while R 2 died on the fifteenth day

Daily examination of the blood of these rabbits receiving either sodium nitrate or erythrol tetranitrate even in massive doses revealed no inclusion bodies No significant postmortem changes were observed in the hematopoietic organs in the three rabbits which died

EXPERIMENT 4 EFFECT OF ERYTHROL TETRANITRATE UPON THE MONKEY

A *Macacus rhesus* monkey received three 180 mg /kg doses of erythrol tetranitrate by stomach tube on the second fourth and tenth days of observation During daily observations for twenty three days a mild anemia was precipitated without the development of inclusion bodies from which there was prompt recovery with cessation of the drug

EXPERIMENT 5 EFFECT OF SODIUM NITRATE AND ERYTHROL TETRANITRATE UPON THE CAT

More suggestive results were obtained in experiments with cats Inclusion bodies appeared within twenty four to seventy two hours in the red blood cells of all cats receiving either sodium nitrate or erythrol tetranitrate

C 1 received a single subcutaneous injection of 1000 mg /kg of sodium nitrate and C 2 injections of 500 mg /kg on two consecutive days In both inclusion bodies up to 9 and 10 per cent appeared within forty-eight hours after the initial dose and then gradually declined as the drug was eliminated

Three cats (C 9 C 3 C 1) received oral doses of powdered erythrol tetranitrate which was thoroughly mixed with raw meat C 9 was given a single dose of 250 mg /kg and showed at seventy two hours 21 per cent of the circulating erythrocytes with typical inclusion bodies and at ninety-six hours 28 per cent with a fall of 2,000 000 in red cells during this period During the ensuing fifteen days the inclusion body erythrocytes gradually declined to zero per cent with a recovery in the red cells In C 3 200 mg /kg of erythrol tetranitrate was administered on the fifth sixth eleventh and twenty-seventh days of observation and 300 mg /kg on the fifteenth and seventeenth days A rare red cell containing inclusion bodies 1 per cent was observed within twenty four hours of the original dose A peak of 15 per cent was attained after the last dose associated with a drop of 1 000 000 in the total circulating red blood cells

In C 1 weighing 2 8 kg erythrol tetranitrate was administered beginning nine days after one dose of 1000 mg /kg of sodium nitrate The inclusion bodies had risen to 10 per cent within forty-eight hours of the sodium nitrate dosage and had gradually fallen again to 2 per cent by the time the first dose of erythrol tetranitrate (150 mg /kg) was administered The next day another 150 mg /kg of erythrol tetranitrate was given followed by doses of 75 mg /kg on the 3 4 5 6 11 and 12 days of observation The inclusion bodies rose to 26 per cent within the first seventy two hours of the erythrol tetranitrate administration and varied from 11 to 28 per cent through the thirteenth day The hemoglobin decreased from 13 3 to 11 2 Gm during the period With discontinuance of the drug on the twelfth day the inclusion bodies declined to 5 per cent by the sixteenth day

In the cats receiving sodium nitrate or erythrol tetranitrate the concentration of inclusion bodies did not vary directly with the dosage of the drug under the conditions of the experiment With the cessation of the drug the number of inclusion bodies was noted to decrease promptly and to disappear entirely eventually A decrease of between one and two million in the red cell count was

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EXPERIMENT 6 EFFECT OF MANNITOL HEXANITRATE ON CAT BLOOD

Less spectacular results were observed in two cats (C 6 C 10) fed mannitol hexa nitrate pulverized in meat Despite relatively large doses of the drug (165 to 330 mg /Kg) only small percentages of inclusion bodies (1 to 6 per cent) were observed in C 10 and the red cell count showed no significant change

An *old* cat (C 6) with an occasional inclusion body erythrocyte (0 5 per cent) even prior to nitrate administration developed a maximum of 12 per cent of these after nine successive doses of mannitol hexanitrate (80 to 160 mg /Kg) the loss in circulating red cells totaling 4 000 000 hemoglobin 3 Gm over a thirty four day period of observation The ra e occurrence of inclusion bodies in the red cells of older animals has been reported elsewhere in the literature [5]

EXPERIMENT 7 EFFECT OF SULFANILAMIDE UPON CAT RED CELLS

Sulfanilamide was only questionably effective in the production of inclusion bodies under the conditions of this experiment

In C 2 which had shown up to 9 per cent inclusion bodies with two 500 mg /Kg doses of sodium nitrate the highest concentration of inclusion bodies observed after ten daily 400 mg /Kg (oral) doses of sulfanilamide was 4 per cent Results were negative in an *old* cat C 7 which showed no significant increase in inclusion bodies over a 5 per cent concentration demonstrable prior to sulfa drug administration nine successive doses of sulfanilamide (800 to 1600 mg /Kg per dose) The red cell count and hemoglobin showed no consistent trend during the period of observation in either cat

STAINING PROPERTIES OF THE INCLUSION BODIES IN CATS

The staining properties of the inclusion bodies observed in the erythrocytes in cats appeared to be similar to those in our patient The inclusion bodies were seen readily in unstained preparations and in supravital preparations stained with brilliant cresyl blue methylene blue and Janus green with neutral red (fig 7) They were also seen but less readily in fixed preparations with brilliant cresyl blue They were not apparent in preparations stained with Wright Wright Giemsa or with the Prussian blue reaction

Certain differences were noted in the cellular reaction in the cats as contrasted with the patient In the animal studies only a minor degree of poikilocytosis and aniso-

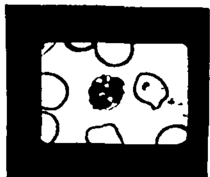
Figs 1 2 Brilliant cresyl blue supravital staining of the erythrocytes in patient s blood Incl sion bodies as seen in bright field under o l imm-rs on

Figs 3 4 5 Similar prepar tions of the peripheral blood from the patient as seen under dark field o l imm rsion conditions Nor- h ghly r-f ctil inclusion bodies Figure 4 shows a separated inclusion body indicating the integrity of thes H inz inner korpern apart from the erythrocytes Figur 5 id ntifi-s th periph-ral locat on of th s bodies at th cell surface

Fig 6 A bright field illustr tion of inclusion bodies from the pat ent s blood Compare and contrast with sim lar bod es appearing in th blood of Cat 4 1 following erythroltetranitrat- oral medication

Figure 7

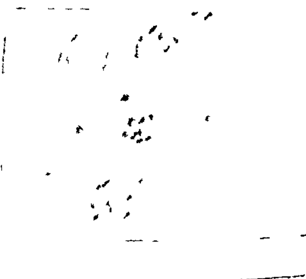

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Figs 1 2 B ill ant cresyl blue supravital staining of the erythrocytes in patient s blood Inclusion bod es as s en in bright fi ld under oil immersion

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F G 6 A bright field illustration of inclusion bodies from the patient s blood Compare and contrast with similar bodies appearing in the blood of Cat # 1 follo ing erythroltetranitrate oral med cation

Figure 7

cytosis was noted, whereas in the human blood, these changes were extreme Per haps this reflects the more profound hematologic disintegration in the patient du to the much longer period of exposure to the drug It was also observed that the inclusion bodies in cat blood tended to remain globular while inclusion bod ies in the patient s blood were more bizarre in shape (figs 6-7) In addition the inclusion bodies observed in the cats under conditions of the experiment oc curred in fewer numbers within each red cell than those observed in the patient

The inclusion bodies both in the patient and in cats were noted in various phases of extrusion from the red blood cell (figs 4 5 7) They were found well within the cell close to the periphery, producing a bulge in the red cell membrane and also lying free outside the red cell Inclusion bodies in both human and cat blood were found to be lysis resistant

A specimen of cat blood, demonstrated to contain inclusion bodies was laked and selectively centrifuged to obtain a highly concentrated specimen of these bod ies Spectroscopic examination of this specimen revealed no hematoporphyrins This evidence while suggestive is not conclusive

Bone marrow examinations were made in cat # 1 after erythrol tetranitrate in massive amounts Unlike the bone marrow of the patient who had received th s drug in small doses over a long period of time the erythroid elements showed no maturation arrest and appeared normal except for the occurrence of small puncti form perinuclear inclusions in a few normoblasts

Discussion

Peculiar inclusion bodies were observed in the red blood cells of a 71 year old male physician who exhibited a severe anemia which did not respond to either intensive iron or liver therapy nor to a high protein high vitamin diet Although folic acid as such[6] was not available for treatment large amounts of vitamin B complex were given There was no clinical evidence of any specific nutritional de ficiency and there was no response to liver The inclusion bodies were noted in the unstained preparation and were stained readily with supravital wet mount technic using brilliant cresyl blue and other reticulocyte stains They appeared as blue green irregular globules occurring singly and in numbers within the mature red cells and taking a position at the periphery They were also seen in various stages of extrusion from the red cells and were noted to be hemolysis resistant The inclusion bodies were distinguished from Howell Jolly bodies and from siderocyte inclusions These various staining and morphologic characteristics were found to conform with those of the Heinz Inner Korpern described in the German litera ture[1-4 7] as occurring in toxic anemias in man and in animals [1-4 -1]

The suspected etiologic agent erythrol tetranitrate which the patient had taken for angina pectoris over a period of one year was administered to cats in massive doses Lysis resistant inclusion bodies with staining characteristics similar to those found in the patient s blood were induced A tendency toward anemia re versible under the conditions of the experiment with cessation of the drug was observed Inclusion bodies were noted to a lesser extent with sodium nitrate and mannitol hexanitrate Only minimal concentrations of inclusion bodies were

produced with large doses of sulfanilamide We can confirm the report of German investigators[8] that old animals show these inclusion bodies in a small proportion of circulating erythrocytes in the absence of any known external toxic agent

Although erythrol tetranitrate and sodium nitrate were given in large repeated doses to rabbits inclusion bodies were at no time observed In a monkey receiving erythrol tetranitrate a mild reversible anemia developed without demonstrable inclusion bodies

Cessation of drug administration to cats brought about a gradual disappearance of the inclusion bodies from the blood stream Whether a longer course of drug administration would have produced an irreversible inclusion body anemia in the cat such as that observed in our patient remains unanswered

The observance of a progressive refractory inclusion body anemia in an elderly patient persisting even after four months omission of the suspected toxic drug would suggest the precipitation of an irreversible toxic alteration in the erythrocyte maturation process

Various theories have been proposed to explain the formation nature and significance of these inclusion bodies Freifeld[8] suggests a genetic relationship between the so-called Randkorperchen (corpora marginalia) and these Inner Korpern of Heinz and Ehrlich the result of an enterogenous autointoxicant These inclusion bodies were originally regarded as dead toxic protoplasm [1,2] reflecting methemoglobin or sulph methemoglobin poisoning [9] The more recent interpretation of these Inner Korpern as denatured cell membrane proteins[1,10] has received support from Jung s observations[7] with the electron microscope which place these inclusion bodies definitely in the outer layer of the cell Frank H J Figge Associate Professor of Anatomy University of Maryland has studied the experimental production of Heinz body erythrocytes[18] When certain sulfonamides were dissolved in water administered to mice large numbers of refractile bodies appeared in the erythrocytes These bodies were insoluble in distilled water or 3 to 5 per cent acetic acid and made leucocyte counts difficult They were similar to erythrocyte inclusions described originally by Heinz Other investigators concur in this identification It was found that a 0 3 per cent sulfanilamide solution given as drinking water induced Heinz bodies in at least 90 per cent of all erythrocytes within four to six days while sodium sulfathiazole did not The tendency of various sulfonamides to produce Heinz bodies appeared to parallel the tendency to induce hemolytic anemia Further studies on the physical and chemical properties revealed that these bodies are globules of either denatured hemoglobin or cathemoglobin Erythrocytes extrude these bodies as they form so that large numbers of Heinz bodies accumulate in the plasma as the erythrocytes become hypochromic Heinz bodies are most easily observed in unstained unmounted blood smears and disappear when examined in oil balsam or other mounting media These globules of hem containing protein denatured within the cell by drugs have been studied in detail because such a reaction is of interest both from the standpoint of cancer research and the mode of action of sulfonamide drugs Dr Figge in a recent personal communication[19] further states I still do not know the exact mechanism which is responsible for the production of these bodies They can be produced in

small numbers by such diverse agents as cobalt paraminobenzoic acid and acetanilide I suspect that they are probably formed as a result of therapy with numerous other compounds but go undetected because blood is usually prepared for examination with some mounting medium As you have probably noticed these bodies are much more easily observed in plain dried blood films examined under the high dry objective

The conception of Heinz Inner Korpern as nuclear fragments has not been confirmed though Figge states this protein which is denatured resembles in some respects a nucleoprotein

Schilling who noted all transitions between typical Howell Jolly bodies and the Inner Korpern following splenectomy [9] rejected this simple explanation [11] The appearance of inclusion bodies in the peripheral blood of various normal animals following splenectomy [2 8 9 11] and of humans with splenic hypoplasia subsequently demonstrated [2] bears out the hypothesis of Heinz[1] and of Schilling[9] that the normal spleen filters out the senile and damaged erythrocytes which may contain these inclusion bodies This is further corroborated by the observation of inclusion bodies (Giemsa stained Tupfer preparation) as partly extra-cellular and partly extruding from the red cells in the human spleen [2] It is suggested that in the absence of a normally functioning spleen the inclusion containing red cells may appear in the peripheral blood The administration of a specific toxic agent further increases their numbers [3]

Should it be considered then that minimal inclusion body formation may occur in association with subclinical endogenous toxins, in otherwise normal individuals but in numbers so small that they are ordinarily withdrawn by a physiologically functioning spleen? This concept might explain the more frequent observation of small numbers of inclusion-containing erythrocytes in the blood stream of senile animals [8] animals in whom the catabolic processes predominate and splenic efficiency perhaps may have diminished In addition there may be other states such as dietary deficiencies inherent specific susceptibilities and certain disease processes which perhaps may predispose to inclusion body anemia One may also inquire as to whether the development of inclusion bodies may precede red blood cell destruction at some phase in anemic states other than those of toxic etiology It has been shown that in poisoning with nitrates and their derivatives the extent of inclusion body development parallels closely the destruction of the red blood cells [4]

The known toxic agents which may result in an inclusion body anemia in man and in animals are in general methemoglobin producing drugs such as nitrobenzol aniline [8 9] nitroglycerine [4] dinitroglycol [4] ethyl nitrate [4 12] sodium nitrate [1] nitrobenzol and toluol derivatives [8 13] Even in the absence of a known toxic agent as in an hemolytic anemia observed in rats following splenectomy inclusion bodies have been reported in association with methemoglobin formation [8]

Nevertheless a cause and effect relationship between methemoglobin and inclusion body erythrocytes has been questioned The appearance of inclusion bodies in the peripheral blood does not always follow methemoglobin formation [8 14]

In one investigation the agent rather than the methemoglobin per se was found to be the more important factor in the concentration of inclusion bodies in the blood Thus the nitrates which form less methemoglobin than the nitrites have produced many more inclusion bodies [12]

Observations[3] in eleven patients with acute poisoning from aniline or nitro-derivatives have revealed that inclusion bodies appear only several hours after methemoglobin is formed Moreover several more hours may elapse as noted in a schizophrenic who had swallowed one half glass of aniline before these bodies become prominent and involve a maximum number of the mature circulating red cells even to 100 per cent In this patient massive hemolysis of the red blood cells was noted on the fifth day and inclusion bodies were seen extruding in various stages from hemolyzing red blood cells Immediately after there occurred a reticulocyte crisis with normoblastosis [3]

Since 1943 there have appeared several reports in the German literature of sulfonamide drug anemia preceded by the appearance of inclusion containing erythrocytes [14-17] It has been advised that if more than 20 per cent appear a severe hemolytic anemia may be forecast and the drug should be forthwith discontinued [14]

Summary

1 Inclusion bodies distinguishable from the Howell Jolly bodies were observed in the red blood cells of a patient with a severe refractory fatal anemia who had been receiving erythrol tetranitrate over a period of one year

2 Bodies with similar staining characteristics were reproduced in cats with large oral doses of erythrol tetranitrate and other nitrates These were generally accompanied by a temporary fall in the red cell count followed by recovery upon withdrawal of the drug

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small numbers by such diverse agents as cobalt paraminobenzoic acid and acet anilide I suspect that they are probably formed as a result of therapy with numerous other compounds but go undetected because blood is usually prepared for examination with some mounting medium As you have probably noticed these bodies are much more easily observed in plain dried blood films examined under the high dry objective

The conception of Heinz Inner Korpern as nuclear fragments has not been confirmed though Figge states this protein which is denatured resembles in some respects a nucleoprotein

Schilling who noted all transitions between typical Howell Jolly bodies and the Inner Korpern following splenectomy [9] rejected this simple explanation [11] The appearance of inclusion bodies in the peripheral blood of various normal animals following splenectomy[3 8 9 11] and of humans with splenic hypoplasia subsequently demonstrated [2] bears out the hypothesis of Heinz[1] and of Schilling[9] that the normal spleen filters out the senile and damaged erythrocytes which may contain these inclusion bodies This is further corroborated by the observation of inclusion bodies (Giemsa stained Tupfer preparation) as partly extra cellular and partly extruding from the red cells in the human spleen [2] It is suggested that in the absence of a normally functioning spleen the inclusion containing red cells may appear in the peripheral blood The administration of a specific toxic agent further increases their numbers [3]

Should it be considered then that minimal inclusion body formation may occur in association with subclinical endogenous toxins in otherwise normal individuals but in numbers so small that they are ordinarily withdrawn by a physiologically functioning spleen? This concept might explain the more frequent observation of small numbers of inclusion containing erythrocytes in the blood stream of senile animals [8] animals in whom the catabolic processes predominate and splenic efficiency perhaps may have diminished In addition there may be other states such as dietary deficiencies inherent specific susceptibilities and certain disease processes which perhaps may predispose to inclusion body anemia One may also inquire as to whether the development of inclusion bodies may precede red blood cell destruction at some phase in anemic states other than those of toxic etiology It has been shown that in poisoning with nitrates and their derivatives the extent of inclusion body development parallels closely the destruction of the red blood cells [4]

The known toxic agents which may result in an inclusion body anemia in man and in animals are in general methemoglobin producing drugs such as nitrobenzol aniline [8 9] nitroglycerine [4] dinitroglycol [4] ethyl nitrate [4 1] sodium nitrate [1] nitrobenzol and toluol derivatives [8 13] Even in the absence of a known toxic agent as in an hemolytic anemia observed in rats following splenectomy inclusion bodies have been reported in association with methemoglobin formation [8]

Nevertheless a cause and effect relationship between methemoglobin and inclusion body erythrocytes has been questioned The appearance of inclusion bodies in the peripheral blood does not always follow methemoglobin formation [8 14]

In one investigation the agent rather than the methemoglobin per se was found to be the more important factor in the concentration of inclusion bodies in the blood Thus the nitrates which form less methemoglobin than the nitrites have produced many more inclusion bodies [1]

Observations[8] in eleven patients with acute poisoning from aniline or nitro-derivatives have revealed that inclusion bodies appear only several hours after methemoglobin is formed Moreover several more hours may elapse as noted in a schizophrenic who had swallowed one half glass of aniline before these bodies become prominent and involve a maximum number of the mature circulating red cells even to 100 per cent In this patient massive hemolysis of the red blood cells was noted on the fifth day and inclusion bodies were seen extruding in various stages from hemolyzing red blood cells Immediately after there occurred a reticulocyte crisis with normoblastosis [8]

Since 1943 there have appeared several reports in the German literature of sulfonamide drug anemia preceded by the appearance of inclusion containing erythrocytes [14–17] It has been advised that if more than 20 per cent appear a severe hemolytic anemia may be forecast and the drug should be forthwith discontinued [14]

Summary

1 Inclusion bodies distinguishable from the Howell Jolly bodies were observed *in the red blood cells of a patient with a severe refractory fatal anemia* who had been receiving erythrol tetranitrate over a period of one year

2 Bodies with similar staining characteristics were reproduced in cats with large oral doses of erythrol tetranitrate and other nitrates These were generally accompanied by a temporary fall in the red cell count followed by recovery upon withdrawal of the drug

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PART IV

BLOOD CLOTTING PHENOMENA AND HEMORRHAGIC DISEASE

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HISTORICAL NOTES ON BLOOD PLATELETS

By Leandro M Tocantins M D

ON MARCH 7 1842 at a session of the Academie des Sciences of Paris during an account of studies on the microscopy of blood Donne[1] stated in clear terms that there existed in the blood red and white globules and little globules (globulins) There seems to be no earlier record of the recognition of the platelet as a formed element of the blood Later in his book Cours de Microscopie Donne[2] repeated and amplified his original description Although his interpretation of the origin of the globulins (the lymph) was erroneous the substance and manner of his opening statement indicates that he had clearly recognized the existence of a distinct morphologic element (fig 1) Signs of awareness of the presence in the blood of forms other than white and red cells were evident before Donne In Hewson s and Andral s[3] works references are made to certain white globules and bodies distinct from leukocytes and red corpuscles but of uncertain nature occurrence and identity

At about the same time as Donne Zimmermann described certain bodies which he believed were the precursors of red blood cells He called them Elementar blaschen and remarked on their tendency to gather in clumps Zimmermann[4] appears to have been among the first to use anticoagulants in the cytologic study of blood He bled a horse and collected the blood in a solution of 6 per cent magnesium sulphate equal parts of blood and solution In this mixture he found what were undoubtedly platelets

Among the first attempts to attribute the origin of platelets to other blood elements is the work of Schultze[5] (1865) He had observed in normal blood small elements which he considered to be of protoplasmic nature These had a strong tendency to clump and form granular masses (Kugel) which he did not consider identical with Zimmermann s elementar blaschen he thought they resulted from the destruction of white corpuscles Schultze s hypothesis was corroborated in 1872 and subsequent years by Riess [6] who maintained that during anemias and cachectic states the white corpuscles fragment and give rise to smaller corpuscles which he called zerfallskorperchen (disintegration bodies) analogous to the Kugel of Schultze but differing from Zimmermann s Elementarblaschen

Throughout the third quarter of the past century one finds here and there what were probably platelets described as bacteria [7] The morphologic variability of platelets depending on the conditions of collection and observation of the blood was perhaps the reason for regarding them as extrinsic matter peculiar to certain pathologic conditions In 1873 Vulpian[8] noted the presence in the blood of colorless corpuscles having the properties of sticking to the cover glass and accumulating in clumps In the same year Ranvier[9] observed in the center of the fibrinous network that appears during coagulation of blood granulations with tinctorial characteristics different from those of leukocytes and erythrocytes He advanced the

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them hematoblasts Hayem attributed to the hematoblast a double function it was a coagulation accelerating agent (le sang porte dans son sein un hemostatique puissant)[1] and it played a role in the regeneration of blood a conclusion based on his observations on changes in the platelets after hemorrhage and in acute diseases Some of Hayem s first studies were concerned with the evolution of the red blood cells in the blood of oviparous and viviparous vertebrates It was these and subsequent studies that led him to consider the platelet as a precursor of the red blood cell Without the breadth of his training and experience it was and is difficult to follow his views Today the available evidence makes it unlikely that such a relationship between platelets and red blood cells exists as claimed by Hayem The prevailing view of the origin of platelets renders it improbable that a body that arises from fragmentation of the cytoplasm of megakaryocytes may later be transformed into a red blood cell Moreover the morphologic features of the precursors of red blood cells are now well e ablished and differ substantially from the platelet

The existence of the platelet as a distinct element in blood within the vessels was further confirmed by Bizzozero [13, 14] He seems to have been the first to observe the platelet circulating in the blood of living animals He stressed that the granular masses of Schultze were neither residues of white corpuscles which were destroyed before or after collection of the blood nor granulations of fibrin as thought by Ranvier The masses were derived from special morphologic elements pre existing in the blood which he called plattchen Bizzozero was probably influenced by the expression blaschen (globule vesicle) that had been previously used by Zimmerman It was Bizzozero[13] who established securely the foundation for the present day conception of the platelet as a distinct element of the circulating blood (Einen neuen Formbestandteil des Blutes)(1882) and indicated the part it plays in thrombosis Before his work the white thrombi were considered to be made up principally of leukocytes [15] By a number of ingenious experiments Bizzozero demonstrated that the white portion of these thrombi consisted almost exclusively of platelets gradually accumulated at a point in the vessel where the wall had been injured or the circulation obstructed Once accumulated in a mass in vivo or in vitro the platelets underwent changes in appearance became unusually sticky a phenomenon he designated as viscous metamorphosis Bizzozero s observations had the merit of having been made on the circulating blood of a living animal (fig 3) Osler s observations were made on the vessels of dead animals it was perhaps because of this fact that at the time of his report Osler[7] was not quite clear in his mind whether these bodies might be bacteria The title of his paper An Account of Certain Organisms Occurring in Liquor Sanguinis [10] carried that implication The expression plaquette was not used by Bizzozero[16] in his work until 1891 and seems to have been introduced by Hayem[17] in 1883 Bizzozero originally referred to these bodies as petites plaques or simply plaques (fig 3) The English word platelet does not seem to have been used until later in the 19th century Osler[18] was among the first to translate Bizzozero s expression Blut Plattchen as Blood Plates although he did not consider it a good descriptive word Other expressions used to designate these elements were blood plaques

view that these masses probably determine the coagulation of blood very much in the same way as the crystal of a salt brings about the crystallization of a saturated solution of that salt

In 1874 Osler[10] pointed out that the granular masses of Schultze result from the agglutination of small bodies *which occurred as single units in the circulation* He demonstrated and his illustrations clearly showed for the first time that these granular masses occurred as single elements in the circulating blood (fig 2) and

PHYSIOLOGIE.—*De l'origine des globules du sang, de leur mode de formation et de leur fin, par M. AL. DONNÉ.* (Extrait par l'auteur.)

(Commissaires MM. Magendie, Flourens, Dumas, Milne Edwards, Payen.)

Il existe dans le sang trois espèces de particules: 1° les globules rouges ou sanguins proprement dits, 2° les globules blancs qui n'ont été bien connus que dans ces derniers temps, 3° les globulins du chyle.

Les globules rouges sont plats dans toutes les espèces de sang; ils sont circulaires dans le sang des mammifères, et elliptiques dans celui des oiseaux, des poissons et des reptiles.

FIG 1 EXCERPT FROM DONNÉ'S REPORT BEFORE THE PARIS ACADEMY OF SCIENCES (1842)

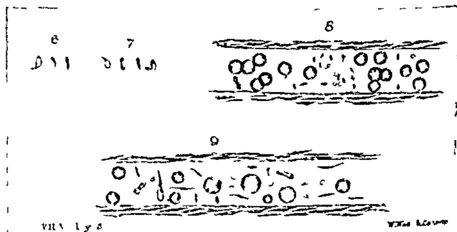


FIG 2 DRAWINGS BY WILLIAM OSLER ILLUSTRATING THE ORGANISMS HE OBSERVED IN THE VENULES OF FRESHLY KILLED RATS (1874)

came together when the blood was shed Osler's observations were carried out in the venules of recently killed young rats and mark the time when the platelet began to be held not as an artefact or a by product of a change in shed blood but as a normal constituent of the circulating blood itself

A few years later Hayem[11] pointed out that il existe dans le sang de tous les vertebres des petits elements qui ne sont ni des hematies ni des globules blancs He however believed that the elements represented primitive red corpuscles that had not gone beyond a certain phase in their evolution for this reason he called

the view held by Schmidt [5] that the fibrin ferment originated from the destruction of leukocytes Among the opponents of Bizzozero s ideas were Weigert [28] who at tributed the findings of the Italian investigator to artefacts resulting from vessel compression circulatory disturbances and the anesthesia employed Bizzozero countered these objections by repeating and confirming his own observations on the *intact* vessels of the wing of a living unanesthetized bat [16]



Fig 1 Normaler schneller Blutstrom axialer Character

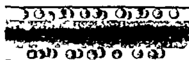
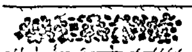


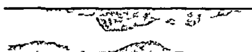
Fig 2 Ran lstell ng der Le cocyten geri ge Verlangsamung *der Circulation geschwi d gkeit.*



F g 3 Bl tplättchen a in der plasmatisch n Randzone Sta ke Verlang m ng der Circulatio sg chwind gkeit. Abnahme der Randstellung der Le cocyte



F g 4 St gnat on N ch A b aline roth Thrombo e ach B bin Comm nic t mit ei m st ömen l fä se Plättchen a z m Theil e ä dert (zack g).



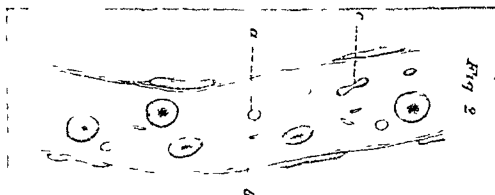
F 5 Wandständ ger Blutplättche thromb s.

Fig 4 Illustrations of the Paper by Eberth and Schimmelbusch Depicting the Mode of Formation of Platelet Thrombi (1885)

Wooldridge[29] on the basis of experiments with incoagulable bloods especially after the injection of peptone believed that platelets were simply a precipitate of the globulin portion of the plasma (Globulin Plattchen) and not a distinct element of the blood Lowit[27] supported Wooldridge s contention and brought out much evidence against the existence of the platelet The idea that the platelet as such does not exist in the blood is still encountered in modern texts [30] a reper cussion of the observations of Wooldridge The observations of Bizzozero were later confirmed and extended by Eberth and Schimmelbusch[31] and Laker[32] while

disklets [19] third corpuscles [20] [21] fugitive corpuscles fugitive discs invisible colourless discs [2] [3]

Among those who lent their support to the discovery of the new morphologic element was William H Howell who died in February 1945 after a career in physiology extending over a half a century His first paper corroborating and extending Bizzozero s observations was published in Science in 1884 [4] One of his last



En examinant avec un objectif à immersion le contenu de ces vaisseaux (veines ou capillaires) on arrive à ce résultat surprenant qu en réalité *à coté des globules rouges et des globules blancs circule un troisième élément morphologique* (fig 2) Il est représenté par de petites plaques très pâles de la forme de disques à surfaces parallèles ou plus rarement, de lentilles ovales ou rondes d un diamètre egal au tiers ou à la moitie de celui des globules rouges Ces plaques sont toujours incolores et circulent dispersées irrégulièrement entre les autres globules ne montrant point de préférence pour la partie centrale plutôt que pour la partie périphérique du courant Ordinairement elles sont isolées les unes des autres ce qui n empêche pas cependant que souvent on ne les trouve réunies en groupes plus ou moins grands Cette agglomeration est déjà

FIG 3 ILLUSTRATION AND TEXT IN BIZZOZERO S PAPER DEMONSTRATING THE EXISTENCE OF PETITES PLAQUES IN BLOOD WITHIN THE VESSELS OF LIVING ANIMALS (1882)

papers proposing the lungs instead of the bone marrow as the principal source of platelets appeared in 1937 [5]

Bizzozero s monograph gave rise to much opposition from the pupils of A Schmidt [6] and Lowit who adhered to the theory that the bodies designated as Plattchen by Bizzozero were derived from fragmentation of leukocytes Much of the disagreement and contradiction in papers that followed Bizzozero s may be attributed to technical difficulties that even today try the patience of those engaged in the study of platelets The many shapes and arrangements presented by these bodies have confused some investigators whose opinions rested mostly on morphologic evidence obtained in vitro Bizzozero also departed sharply from

mechanism of hemostasis A year later (1883) Krauss[33] reported in one form of hemorrhagic disease purpura hemorrhagica a diminution in platelets followed by an increase after the hemorrhage ceased In 1887 Denys[34] confirmed this finding and later Hayem[35] actually counted only 62 000 platelets per cmm of blood in a young patient with purpura Hayem[35, 36] also drew attention to the large size of the platelets and the soft quality and poor retractility of clots formed from the blood of these patients and attributed these defects to the decrease or absence of platelets

Hand in hand with the efforts of observers of the time in affirming or denying the existence of the platelet as a distinct element went attempts to trace the source of the elusive bodies Many held the view that they originated either from nucleated red cells or from the red cells themselves Engel[37] derived the platelets from the nuclei of normoblasts and Wlassov[38] and Bremer[39] believed they came from disintegrated erythrocytes and did not accept them as constituting a third element of the blood The bodies examined by Wlassov and Bremer must indeed have been by products of disintegration of erythrocytes resembling platelets only in form the same bodies were later studied by Arnold[40] in intravascular clots and in blood allowed to stand outside the body for several hours These ideas were corroborated and in part amplified by Muller [41] Determan [42] Maximow [43] and Schwalbe [44] Antagonists of the theory that the platelet was derived from the de struction of red blood cells were Petrone [45] Sacerdotti[46] and Dominici [47] who criti cized the view of Arnold and adherents of his theories and pointed out that their conclusions rested on artefacts from preparations of dried smears of blood This recurring source of confusion was partly due to the fact that differentiating stains were at the time not generally available It was not until the polychrome Ro manowsky stains and azure dyes began to be widely used that it was possible to separate the red to violet azurophilic granules of the platelet from all sorts of granular material in and out of cells Dominici[47] introduced a new and what proved to be a partially correct conception of the origin of the platelet He held that plate lets were organites that is formed elements liberated by cells and lacking a nucleus As the mother cells of the platelets he described mononuclear cells with a protoplasm distributed in long pedicles which when broken off made up the platelets It is possible that this conception influenced the work of Wright [48] It differs from his theory in only one respect Wright showed that this fragmentation took place from the cytoplasm of the megakaryocyte

Two other workers of the Italian school came close to the solution of the prob lem of the origin of platelets While studying the formation of the red blood cells Foa and Salvioli[49] observed that the giant cell of the bone marrow first described by Bizzozero[50] in 1869 and later (1890) called megakaryocyte by Howell [51] frag mented into many colorless hyaline bodies Foa and Salvioli thought these cell fragments were the precursors of the red blood cells This view and Hayem s ideas overlap at certain points for Hayem thought that platelets (which we now know were the cell fragments observed by Foa and Salvioli) were the precursors of eryth rocytes (hematoblasts)

Bizzozero s conception of the platelet as an independent element of the blood

studying the circulating blood in the vessels of living dogs and other mammals Eberth and Schimmelbusch further pointed out that in blood within the vessels the corpuscles usually run in the center of the stream the periphery being made up of plasma alone (fig 4) Slowing or stasis of the circulation is followed by migration of leukocytes and platelets to the periphery of the stream while red cells remain in the center In one particular Eberth and Schimmelbusch differed from Bizzozero He believed *that coagulation of the blood followed changes in the platelets* while they considered the two phenomena concurrent but independent of each other Eberth and Schimmelbusch described in the blood of oviparous animals nu

Désignations	Hématoblastes	Hématies	Globules blancs
Nouveau né de 24 heures	2[illegible]2 000	5 0[illegible] 000	1[illegible] 000
— de 3 jours	[illegible]00 000	[illegible] 138 000	7 [illegible]0
— *de 8 jours*	[illegible]16 000	5 487 000	9 000
— de [illegible] jours	231 000	[illegible] 0[illegible] 000	11 000
— de 13 jours	317 000	[illegible] 000	11 300
Enfant de 8 mois non sevré	31[illegible] 000	3 [illegible]00 000	11 1[illegible]
— de 9 mois »	[illegible]76 000	4 [illegible] 000	1 3[illegible]0
— de 15 mois 1/2 sevré	318 000	3 [illegible]06 000	10 000
— de 4 ans	[illegible]60 000	5 1[illegible]7 000	12 000
Homme de 25 an	231 000	[illegible] 487 000	5 [illegible]00
— de 36 ans	231 000	3 979 000	6 830
— de 72 ans	318 000	4 [illegible]91 000	7 700
Femme de 26 ans	231 000	4 557 000	[illegible] 300
— de 32 ans	2[illegible]0 000	4 27[illegible] 000	6 800
— de [illegible] ans	[illegible]16 300	5 363 000	5 6[illegible]

Fig 5 Table Giving the Result of the First Accurate Platelet Counts by Hayem (1878)

cleated bodies (spindeln spindle cells) that underwent changes similar to those undergone by platelets during coagulation

Attempts to count the number of platelets in the blood seemed to have been made first in 1872 by Riess [6] Technical difficulties must have interfered with the accuracy of his determinations for Riess found increases in the number of platelets in diseases which we know today are accompanied by thrombopenia He did however observe that in pernicious anemia there was a diminution a point later confirmed by Osler [18] We probably owe to Hayem[11] in 1878 the first accurate counts of platelets (fig 5) The figure for the average number of platelets per cubic millimeter of blood generally accepted today as representing the normal does not differ *significantly* from that found by Hayem

From a consideration of the facts brought out by Bizzozero Hayem[1] concluded in 1882 that *the platelet thrombus played an important part in the arrest of bleeding* and that a decrease or absence of platelets should result in disruption of the

when he proposed the bleeding time of the skin as a test of a general tendency to bleed Duke showed that there existed a close correlation between the number of platelets in the blood and the duration of the bleeding time Apparently for the first time abundant clinical as well as experimental evidence was gathered in support of this relationship Duke was responsible perhaps more than any other contemporary author for strengthening the concept of the role and importance of platelets in disorders of hemostasis

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was soon carried even further by those who claimed for it cellular characteristics common to most cells Deetjen[5] using osmic acid fixation believed he had shown that platelets had a nucleus and protoplasm By special technics he also claimed to have demonstrated that platelets send out protoplasmic processes similar to the pseudopods sent out by leukocytes a fact confirmed by Wright [48] who saw some of the pseudopods retract *The observations of Deetjen were supported by* Deckhuysen[53] and Koppsch [54] These concepts influenced Deckhuysen perhaps to regard the mammalian platelet as homologous to the nucleated spindle cells of invertebrates and oviparous animals which behave when the blood is shed very much like the mammalian platelet This is why Deckhuysen called these cells in the lower species *thrombocytes*

Vassale[55] and Foa and Carbone[56] were among the first to find platelets in the spleen Foa[57] differed from Vassale regarding their presence in the lymph nodes



FIG 6 COPY OF A COLOR PLATE IN WRIGHT'S ARTICLE (1910)
Megakaryocytes bordering the sinusoids of the bone marrow and extending processes into the blood current fragmenting giant platelets

Foa considered the hyaline bodies of the lymph nodes to be fragments of mononuclear cells and not true platelets

Wright's discovery in 1906[58] that the megakaryocyte of the bone marrow gave rise to platelets by *fragmentation of its cytoplasm was the next important* development (fig 6) All those present when Wright exhibited his preparations agreed that his demonstration was quite convincing [59] His views however were taken up slowly by European investigators Part of the reason for this might have been the relative lack of clarity of the illustrations accompanying his first contribution (1906) [58] His arguments were strengthened by a later report in which the documentation was clear and striking [48] With but a few exceptions one by one the leading histologists of the world have come to accept his views

It was probably due to Wright's interest and stimulating influence that W W Duke became interested in the problem of platelets and their relation to abnormal bleeding Duke came under Wright's influence early in his career as a house officer in the Massachusetts General Hospital The role of the platelet in spontaneous hemostasis first clearly visualized by Hayem was convincingly shown by Duke[60]

THE ROLE OF ALLERGY IN THE PATHOGENESIS OF PURPURA AND THROMBOCYTOPENIA

By Frederick W Madison M D

ALLERGY by one term or another has been recognized as an etiologic factor in purpura since the earliest descriptions of that disease Until very recently however the intangible nature of both allergy and purpura the multiplicity of potential etiologic factors and the lack of exact knowledge of the pathologic changes in the latter state have made determination of the relative importance of allergy virtually impossible Even now it is very difficult to evaluate the role of the allergic mechanism with accuracy To attempt such an evaluation it is essential to review briefly the chronologic development of the knowledge of the existence of allergic factors in the pathogenesis of purpura It is likewise essential to define terms for as Pines[1] has recently stated *Among the confused chapters in hematology purpura is the most confused*

For a number of years it has been our custom to use the term purpura to indicate vascular changes only[2] These vascular changes are characterized by reversible alterations of as yet unknown character in the walls of the smaller vascular radicles which make possible the escape of whole blood from the vascular bed Such escape may occur more or less spontaneously (petechiae nontraumatic ecchymoses) or it may be induced by increasing the intravascular pressure (e g tourniquet test[3] Gothlin test[4] etc) or by decreasing the extravascular pressure (suction test[5] capillary resistometer[6] etc) Artificial induction of petechiae is the most satisfactory criterion for the diagnosis of purpura at the present time although lack of standardization of technics and variability of the vascular changes have made interpretation somewhat difficult Other tests[7] [8] are more complex and less suited to routine clinical use but are useful for supplementary or corroborative purposes After comparative trial of the various tests we have come to rely almost entirely upon a simple tourniquet test done with a pneumatic arm band at 100 mm of mercury (unless the systolic pressure is below that level in which case it is correspondingly reduced) and *maintained for 8 minutes unless extravasation of* blood is so marked as to cause excessive infiltration of cutaneous tissues in which case it is stopped at a shorter time A representative area 2.5 cm in diameter is chosen and the petechiae in it are either counted or recorded graphically More than ten easily seen petechiae within the circle is regarded as a positive test and the petechiae in excess of that number may be regarded as a quantitative expression of the test A positive test is interpreted to indicate the presence of purpura but a single negative test is not adequate to rule it out because of the variable nature of the vascular changes

Purpura in this sense may and does most frequently exist alone[2] It may however coexist with or be complicated by defects in the coagulation or clot retraction mechanism of the blood (hypoprothrombinemia thromboplastin deficiency thrombocytopenia fibrinogenopenia) in which cases the combination of inadequate blood coagulation and leaky vascular walls produces an hemostatic error of such magnitude as to cause serious blood loss From a clinical standpoint it would seem to be much simpler to separate the vascular and hematologic factors and at least for the purpose of etiologic studies to consider thrombocytopenic purpura as purpura with thrombocytopenia or as two coexisting abnormalities The im

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dominal pain and intestinal hemorrhage Six years[16] later he added several similar cases and the syndrome characterized by purpura and abdominal pain has since borne his name Many years later Glanzmann [17] in reviewing these syndromes suggested that the basic mechanism in all of the cases of both the Schonlein and the Henoch types was of allergic nature and suggested that they be termed an aphylactoid purpura a view which has been almost universally accepted The clinical reports of Osler [18] and more recently of Eyermann[19] and others have firmly established the importance of the allergic mechanism as an etiologic factor in a considerable portion of the cases of simple purpura without coagulation defect It is interesting to note that in 1914 Osler stated that perhaps the anaphylactic key will unlock the mysteries of the purpuras

Thus by the middle of the nineteenth century the multiplicity of etiologic factors in purpura was well recognized It was known that it might occur as a result of or in association with a varied group of clinical states including infections diseases of the blood malignancies cachexia endocrine disturbances deficiency states in addition to gastro intestinal skin and rheumatic syndromes that are now regarded as of allergic nature Clinical reports and experience since that time have confirmed amply the existence of all of these factors and in recent years have emphasized the importance of the allergic group

Until the latter part of the nineteenth century purpura was considered simply as a vascular disease because of the paucity of knowledge of blood coagulation defects There had been no knowledge of the existence of blood platelets until *the studies of Donne [0] (1842) suggested the presence of a third cellular substance* in the blood Hayem[21] confirmed these studies in 1878 and Bizzozero completely established the identity of blood platelets in 1882 Brohm[23] (1881) Denys [4] (1887) and Hayem [5] (1895) soon discovered the fact that the blood platelets were sharply reduced in some cases of purpura though not in all

In the decade or two which followed the discovery of the relation of platelet reduction to purpura profound changes occurred in the interpretation of the dis ease Attention was focused primarily on the platelet reduction and clinical cases were divided into thrombocytopenic and nonthrombocytopenic types with sub division into secondary and primary groups depending upon whether or not they were associated with recognizable clinical disease Partly because of its dramatic clinical manifestations and partly because of the absence of tangible etiologic clues primary thrombocytopenic purpura quickly occupied the center of atten *tion and has retained that position to the present time Numerous synonyms have* developed including essential and idiopathic thrombocytopenic purpura and Morbus maculosis Werlhofii with varying appropriateness It is important to recall also that prior to the time when the concept of primary thrombocytopenic purpura came into existence it was impossible to differentiate severe purpura from hemo philia hypoprothrombinemia and fibrinogenopenia as we know them today Con sequently it is not surprising that thrombocytopenic purpura with its severe and often lethal blood loss should have been grouped with these diseases under the general title of hemorrhagic diseases and more or less separated from its closer allies the nonthrombocytopenic or simple purpuras This confusion was rela

portance of this dual approach is apparent in tracing the chronologic development of the knowledge of the etiology of these states

One of the earliest discussions of the etiologic factors in vascular purpura is to be found in the Opera Omnia of Riverius According to the English translation of Culpeper[9] (1678) he stated But there is one Symptome proper and peculiar to a pestilential feaver which doth not happen in other Feavers viz Purple Specks or Spots on the whole body which the Italian Physicians name Peticulae or Petechiae and these Feavers which have these symptoms are commonly named Purpuratae or Petechiales and sometimes they are very large and possess whole members and then the parts appear tainted with redness which in a few hours oftentimes vanisheth away and then returns again and are commonly called Ebullitions of the blood There do appear in other Diseases spots very like unto those aforesaid but springing from a far different cause viz from the over thinness of the blood which being exagitated by the heat or the expulsive faculty does sprout forth of the Capillary Veins into the Skin These spots are wont for the most part to appear in such as have some Flux of the Blood because the Blood in such is more thin and watery and also in Splenetick persons and in such as have the Jaundice and old obstructions of the Bowels and in all such who are apt to fall into a Cachexy Interpreted in modern terms Riverius suggested that infections blood diseases malignancy diseases associated with splenomegaly jaundice and cachexia were of etiologic importance in purpura The reference to the redness which in a few hours oftentimes vanisheth away and then returns again may well be construed to represent the earliest recognition of an etiologic mechanism which might now be regarded as of allergic nature

The next important addition to the list of causative or associated factors came a hundred years later when Werlhof[10] in 1735 described the classical case of an adult girl robust without manifest cause attacked toward the period of her menses with a sudden severe hemorrhage from the nose and about the neck and on the arms spots partly black partly violaceous or purple It is well to recall that no knowledge of thrombocytopenia or other coagulation defect existed in the eighteenth century but regardless of the presence or absence of such defect this would seem to be the first recognition of endocrine factors in the etiology of purpura In the same period Hornung[11] suggested that clinical purpuras be divided into simplex febrile and scorbutic types thus apparently recognizing in his third group what we now know to be deficiency states as important etiologic factors

The most tangible early suggestion of the relationship of allergy to purpura is found in the classification of Willan[1] (1808) which included five types purpura contagiosa purpura simplex purpura senilis purpura hemorrhagica and purpura urticans It is of interest to note that this classification may have been the origin of the term purpura hemorrhagica which has continued in general usage to the present time A few years later Schonlein[13] (1837) reported the symptom complex which has borne his name and in which purpura occurred in association with multiple joint involvement The recent report of Montgomery[14] of the incidence of purpura in rheumatic fever has re emphasized the importance of this symptom group In 1868 Henoch[15] described a similar case and recorded the addition of severe ab

many others have amply confirmed prompt and dramatic increase of the circulating platelets following splenectomy in certain cases of thrombocytopenia Interestingly Elliott[35] has also demonstrated prompt reversal of the vascular changes following splenectomy in cases of purpura with thrombocytopenia In the intervening thirty years splenectomy has been established as the standard therapeutic approach to cases of primary or idiopathic thrombocytopenic purpura and has been shown to be particularly effective in those instances in which there is an ample number of megakaryocytes in the bone marrow In spite of that position in therapy however removal of the spleen still remains a somewhat empiric procedure for the mechanism by which it influences the level of the platelets in the circulating blood has never been clarified There is still uncertainty and controversy as to whether the spleen destroys the platelets inhibits their development in the bone marrow or controls their release from the marrow and what relation if any it bears to the allergic mechanism which seems to bear close resemblance to splenic action clinically

Unfortunately methods for the clinical study of the allergic mechanism in the production of thrombocytopenia which has been so convincingly demonstrated experimentally have been relatively unsatisfactory However thrombocytopenia with purpura following ingestion of various drugs has been reported by Loewy[36] Peshkin and Miller[37] and others and thrombocytopenia has been produced at will in some of those patients by readministration of the offending drug and by skin testing thus establishing the reaction as of allergic type Further demonstration of the allergic mechanism responsible for the granulopenia in agranulocytic angina[38] and of the fact that sensitivity could be detected in those instances by granulopenic response following ingestion of the offending allergens has suggested that platelets might behave in a similar manner and has corroborated the soundness of the ingestion method of demonstrating thrombocytopenic response to allergenic substances Utilizing that method of testing in addition to the usual allergic diagnostic methods it has been increasingly possible in recent years to establish allergic reactions as one of the causes of clinical thrombocytopenia with or without purpura Squier and Madison[39] and others have shown that allergenic foods are capable of producing thrombocytopenia by demonstrating platelet reduction following ingestion of those foods and have shown return of the platelet count to normal levels after the removal of the foods from the diet with clinical recovery

Thus it is evident that in the evolution of knowledge of etiologic factors in thrombocytopenia and in purpura allergy has been established as one of several factors capable of producing thrombocytopenia and likewise as one of several similar factors that are capable of producing the vascular changes characteristic of purpura The curious similarity of these etiologic factors may well explain why purpura occurs so much more frequently in association with thrombocytopenia than with hypoprothrombinemia or thromboplastin deficiency In the case of the allergic factor it is readily conceivable that an allergic individual might have both hematologic and vascular response simultaneously and to the same allergen producing typical thrombocytopenic purpura Clinical evidence to support this possibility is accumulating slowly and is derived principally from satisfactory

tively short lived however and was cleared by the development of practical methods for the enumeration of platelets demonstration of the lack of syneresis in thrombocytopenic purpura (Hayem) [5] development of satisfactory methods for determination of coagulation time development of the tests for bleeding time (Duke) [6] and prothrombin time (Quick) [7] and the clinical observations of Hayem [5] Minot [28] Duke [29] and many others

The pathogenesis of the thrombocytopenia which was found in the last decade of the nineteenth century to occur so frequently in association with purpura has been the subject of many experimental and clinical studies since that time although a few workers have maintained interest in the vascular phases of the disease and a still smaller number have steadfastly persisted in efforts to correlate the vascular and hematologic phases Hayem suggested that the reduction of platelets was due to decreased production or increased destruction of those elements but since the origin and fate of the platelets were still unknown at that time he was unable to throw any light on either mechanism Significantly however he did suggest the possibility of an allergic factor in the latter mechanism by demonstrating the reduction of platelets in anaphylactic states after peptone injection and with heterologous serum He also demonstrated platelet reduction in severe infections It was during this period apparently that the concept of the vascular changes being due to the platelet reduction gained favor a concept which contributed much to the confusion of the subsequent years Hayem s experimental work with heterologous serum doubtless provided the background for the tremendous interest in the reduction of platelets by the use of antiplatelet sera which was studied by many observers during the early part of the twentieth century and which demonstrated beyond question the susceptibility of the platelets to antiplatelet substances of biologic origin

It was also during this period that the origin of the platelets in the megakaryocytes of the bone marrow was established by Wright[30] and corroborated by Bunting[31] and by Downey [3] This observation provided a valuable clue to the mechanism by which platelet reduction might occur as a result of decreased production and it was soon established by Duke [29] Minot [5] and others that platelet deficiency did occur in bone marrow diseases such as leukemia and aplastic anemia Duke also confirmed the reduction of platelets in severe infections notably diphtheria and tuberculosis after peptone injection after massive x ray irradiation (Heineke) [33] demonstrated reduction by chemical toxins of the benzol type and most importantly from the allergic standpoint showed experimentally the reduction of platelets as a result of a hypersensitivity mechanism in rabbits sensitized to horse serum Except for our present incomplete knowledge of deficiency of maturation factors and the role of the spleen the etiologic background of thrombocytopenia was as complete in theory at that time as it is today Myelopathy severe infection and toxemia chemical intoxication x ray irradiation and allergic reaction had been established as important mechanisms capable of causing a reduction of platelets in the peripheral blood

It was at that point that the tremendously important role of the spleen in platelet reduction was discovered more or less by chance Kaznelson[34] demonstrated and

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clinical response of both thrombocytopenia and purpura to allergic control without splenectomy With advances in the technic of allergic studies and with wider use of the ingestion method of testing it is likely that more cases will be found to fall into the allergic category and to respond favorably to that method of therapeutic approach

Summary

It has been suggested that, for purposes of etiologic investigation thrombocytopenic purpura be separated into its two component parts thrombocytopenia and purpura and that they be regarded as two coexisting abnormalities rather than as a single disease Historical review of the development of knowledge of the pathogenesis of purpura emphasizes the importance and soundness of this dual approach Both thrombocytopenia and purpura have been shown to have a complex etiologic pattern with multiple potential etiologic factors The curious similarity of these two groups of factors may at least partially explain the frequent coexistence of the two abnormalities in the clinical picture of thrombocytopenic purpura

It has been shown that allergy has long been recognized as an etiologic factor of major importance in both purpura and thrombocytopenia It is logical therefore that it should frequently be an important etiologic factor when the two conditions exist together and it is suggested that when diagnostic methods are more adequate a considerable number of cases of idiopathic thrombocytopenic purpura will fall into that category and will yield therapeutically to a proper allergic approach

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and other conditions In making this differentiation the megakaryocyte plays a part but the other cells play a major role

The bone marrow in idiopathic hemorrhagic purpura is cellular and there is little fat The erythroid myeloid ratio is normal in the majority of instances but there is a tendency toward a relative increase in the nucleated red cells which sometimes approaches a 1 1 ratio The distribution of nucleated cells in the bone marrow

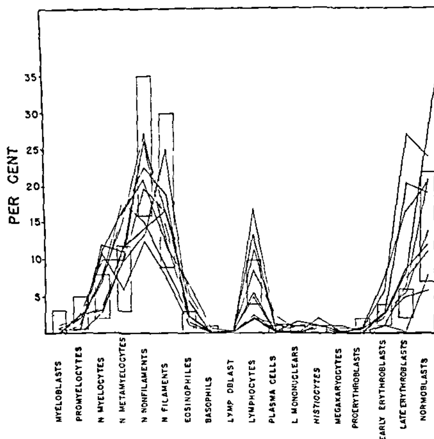


Fig 1 Frequency distribution of nucleated cells in marrow smears of 10 patients with idiopathic hemorrhagic purpura The range of cell in nonhemorrhagic control cases is represented by the shaded areas

smears of 10 of the patients of our series which is representative of the group as a whole is given in figure 1

There is a slight left shift in the maturity of the myeloid cells with the majority of cells at the metamyelocyte to filament stage The eosinophils and lymphocytes are usually normal but may be increased Each of the following cell types un differentiated primitive cells histiocytes mononuclears plasma cells and mega karyocytes constitute less than 1 per cent of the nucleated cells The nucleated red cells are mostly at the late erythroblast normoblast level of maturation and appear normal in size shape and hemoglobin content In patients with chronic purpura who are anemic as the result of blood loss the nucleated erythrocytes

A STUDY OF THE BONE MARROW FROM THIRTY SIX PATIENTS WITH IDIOPATHIC HEMORRHAGIC (THROMBOPENIC) PURPURA

By L W Diggs M D and J S Hewlett M D

IN RECENT summary articles by Nickerson and Sunderland,[1] Rosenthal[2] Tocantins[3] Wiseman Doan and Wilson[4] Limarzi and Schleicher[5] and Dameshek and Miller[6] the extensive literature dealing with idiopathic hemorrhagic purpura is reviewed and the known facts relating to the megakaryocytes and to the bone marrow are presented

The majority of observations have been made on autopsy material and there have been relatively few quantitative studies of material aspirated from the marrow[5, 6] General statements are often made concerning the value of the bone marrow examination in thrombopenic conditions but specific facts are few Because of the paucity of quantitative information a lack of correlation of the marrow findings with prognosis and differences of opinion concerning the number and morphology of megakaryocytes a further study of the bone marrow seems justified

In this paper the observations made at the Cleveland Clinic on the bone marrow smears of 36 patients with idiopathic hemorrhagic purpura and the correlation of the findings with the clinical picture are presented

All of the patients had in common purpura spontaneous bleeding from mucous surfaces platelet counts below 100 000 per cu mm prolonged bleeding time defective clot retraction and normal or only slightly prolonged coagulation time Smears from patients with demonstrable primary disease leukemia aplastic anemia malignancy nephritis cirrhosis or infections or who gave a history of allergy or of taking drugs previous to hemorrhagic episodes were excluded Splenectomies performed on 22 of the 36 patients revealed normal or only slightly enlarged spleens The tissue changes in the spleen were consistent with the diagnosis of essential thrombopenic purpura as defined by Nickerson and Sunderland Unless specifically stated all observations were made on marrow aspirated during the acute hemorrhagic phase of the disease *

The marrow was obtained by needle puncture of the body of the sternum in the midline at the level of the third rib A minimal amount of marrow was aspirated usually less than 0 2 cc Smears were made directly from the point of the needle using the coverslip technic The smears were stained with Wright s stain Smears which contained relatively few nucleated red cells or early myeloid cells and which were obviously diluted with peripheral blood were not included in this study

The Bone Marrow—General

The principal value of the bone marrow examination in thrombopenic purpura is to differentiate idiopathic hemorrhagic purpura from aplastic anemia leukemia

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* Appreciation is expressed to Dr Russell L Haden who performed the majority of sternal aspirations preserved the smears for study and who has allowed us to use the marrow and clinical material for this presentation

TABLE 1.—*The Range and Average Number of Megakaryocytes per 10 000 Nucleated Marrow Cells*

Observer	Method	Megakaryocytes per 10 000 nucleated cells						
		Control			Idiopathic Thrombopenic Purpura			
		Number of cases	Range	Average	Number of cases	Range	Average	Remark
Nickerson and Sunderland	Marrow section	9 normals	24–42	33	4	7–92	38	Postmortem
Limarzi and Schleicher	Indirect slide	10 normals	—	0 59	5	1 4–13 8	7 1	Acute phase
					1	14 9		Chronic phase
Dameshek and Miller	Direct slide	10 normals	0 99–2 →	1 82	5	3 7–7 4	5 2	Acute phase
					6	4 5–15 –	8 4	Chronic phase
Diggs and Hewlett	Direct coverslip	50 misc conditions	1–54	16	36	3–59	17	Acute phase

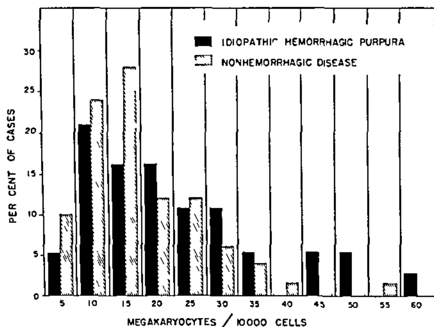


FIG 2 Frequency distribution of megakaryocytes per 10 000 nucleated cells in bone marrow smears from 36 patients with idiopathic hemorrhagic purpura and 50 patients with miscellaneous nonhemorrhagic conditions

Dameshek and Miller state that the average megakaryocyte count for miscellaneous conditions is just over 1 with a range from 0 to 5

may be smaller than normal their cytoplasm more basophilic and their shape irregular Platelets are difficult to find Cells with vacuoles toxic granules and pyknotic nuclei or other signs of degeneration are infrequent

In aplastic anemia the marrow is relatively acellular There is an absolute and relative decrease in myeloid and erythroid elements and a relative increase in lymphocytes mononuclear cells plasma cells and histiocytes The lymphocytes and mononuclears are of a mature variety There are few primitive cells Megakaryocytes are difficult to find The red cells reveal abnormalities in size and shape and there are often atypical and degenerated leukocytes

In smears from patients with leukemia there are numerous cells usually of one variety The normal marrow cells tend to be replaced As a rule there are numerous primitive cells or blasts The megakaryocytes in leukemias at a stage in which there is purpura and thrombopenia are usually significantly decreased

In our experience the bone marrow examination has been of limited value in differentiating primary thrombopenic purpura from thrombopenia secondary to nephritis Hodgkin s disease disseminated lupus or malignancy We have not had opportunity to observe a sufficient number of cases of purpura secondary to chemical poisoning or infections to draw conclusions but degenerative changes in megakaryocytes and in other cells have been described in these conditions

Number of Megakaryocytes

The number of megakaryocytes in relation to other nucleated cells in smears of the bone marrow from normal individuals and from patients with idiopathic thrombocytopenic purpura as reported in the literature are summarized in table 1

In our series the megakaryocyte counts of bone marrow from 36 patients with idiopathic hemorrhagic purpura were made by noting the number encountered while counting 10 000 consecutive nucleated cells All of the counts were made under high dry magnification or oil immersion A tally counter was employed to facilitate counting The number of megakaryocytes ranged from 3 to 59 per 10 000 nucleated cells with an average of 17 (table 1)

Two sternal punctures performed on each of two patients within a period of three weeks during which time there were active hemorrhagic phenomena revealed megakaryocyte counts of 4 and 12 in one case and 8 and 4 in the other

In 50 bone marrow smears from patients with miscellaneous nonhemorrhagic conditions who had no evidence of blood dyscrasias and who had essentially normal marrows the megakaryocytes ranged from 1 to 54 with an average of 16 per 10 000 nucleated cells In this series the megakaryocytes were counted in consecutive fields but the nucleated cells were counted in every tenth field until 1000 cells had been noted The frequency distribution of the megakaryocytes in idiopathic hemorrhagic purpura and in nonhemorrhagic conditions is given in figure 2

The number of megakaryocytes per low power field was estimated in 24 coverslip marrow preparations from patients with idiopathic hemorrhagic purpura Twenty five consecutive low power fields were examined in each case It was found that the number per low power field varies from 0 0 to 8 2 with an average of 1 1

it was noted that there was a marked variation in the platelet response in different patients after operation (fig 3)

Of 22 patients subjected to splenectomy 3 failed to show a platelet rise above 100 000 and 4 sustained a rise of from 100 000 to 200 000 platelets over a three

TABLE 3 —*Megakaryocyte Count in Relation to Prognosis Idiopathic Hemorrhagic Purpura without Splenectomy*

Megakaryocytes per 10 000 nucleated cells	Death	Recurrence	Cure
6			+
8	+		
10			+
10	+		
13	+		
14			+
14		+	
20		+	
27			+
28			+
30	+		
35			+

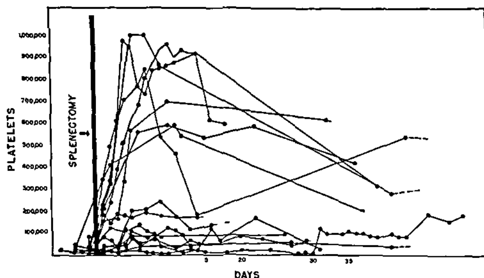


FIG 3 Platelet response following splenectomy in patients with idiopathic hemorrhagic purpura

week postoperative period Six patients responded with a platelet rise of 200 000 to 500 000 and 7 patients with a rise to 500 000 or more over the same postoperative period There was no apparent correlation between the number of megakaryocytes and the platelet count following operation One patient died soon after the spleen was removed One patient was operated upon at another hospital

THE MORPHOLOGY OF MEGAKARYOCYTES

The megakaryocytes were classified as megakaryoblasts immature intermediate

Our observations agree with those found by previous observers that the number of megakaryocytes in idiopathic thrombopenic purpura is within the normal range and that in some cases it is increased beyond the normal average In several cases not included in this series in which the bone marrow smear was not considered satisfactory because of dilution with peripheral blood the megakaryocytes were less than 1 per 10 000 nucleated cells but in all preparations considered adequate for study megakaryocytes were readily demonstrable

Table 2.—*Megakaryocyte Count in Relation to Postoperative Platelet Response and to Prognosis Idiopathic Hemorrhagic Purpura with Splenectomy*

Megakaryocytes per 10 000 nucleated cells	Postoperative platelet response* (3 weeks)	Death	Recurrence	Cure
3		+		
4	++			+
5	+		+	
6	+			+
9	++++		+	
9	++			+
13	+++			+
14	++++		+	
16	++++		+	
16	++			+
17	+			+
19	++++			+
22	++++			+
24	++++			+
24	++	+		
24	+++		+	
29	†	+		
41	+++	+		
43	+++		+	
47	+++	+		
48	+++			+
59	++++			+

* 500 000 or more platelets ++++ 200 000-500 000 platelets +++ 100 000-200 000 platelets ++ less than 100 000 platelets +

† Operation performed elsewhere

The Relation of the Megakaryocyte Count to Prognosis

In order to test the truth of the common belief that patients with high megakaryocyte counts have a good prognosis and will respond favorably to splenectomy whereas those with low counts have a poor prognosis we arranged our patients according to the number of megakaryocytes per 10 000 nucleated cells and tabulated deaths recurrences and cures (tables 2 and 3) It is noted that there is no correlation between the number of megakaryocytes found in the marrow smears during the acute phase of the disease and the prognosis with or without splenectomy

In patients with idiopathic thrombopenic purpura whose spleens were removed

Fig 4 C D fig 5 E F G) This cell is larger than the megakaryoblast being 20 to 50 microns in diameter The shape is irregular and there are varying numbers of cytoplasmic tags and rounded pseudopods The nucleus is indented or divided

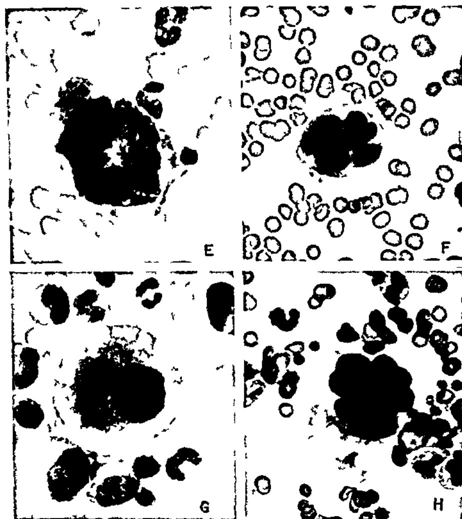


Fig 5 E Immature megakaryocyte with perinuclear granular cytoplasm Spongy nongranular peripheral cytoplasm and pseudopods F Immature megakaryocyte with multilobulated nucleus Granular area next to nucleus Peripheral spongioplasm G Immature megakaryocyte with granules next to nucleus surrounded by a coarsely vacuolated zone and peripheral fine spongy cytoplasm H Intermediate megakaryocyte with ill defined margins Multilobulated pyknotic nucleus Granular cytoplasm Absence of spongy layer at periphery

into two or more lobes and has a moderately coarse chromatin structure without nucleoli The cytoplasm stains blue and is likely to be darker near the nucleus than at the periphery Fine bluish granules are demonstrable next to the nucleus but granules are absent in the peripheral portions and in the pseudopods The pseudopods often contain multiple small vacuoles

or mature megakaryocytes or as naked nuclei The criteria used for the identification of each group are as follows

1 *Megakaryoblast* (fig 4 A B) This cell has a diameter of 15 to 30 microns



Fig 4 A Undifferentiated stem cell ( ) Megakaryoblast B Megakaryoblast in mitosis Nongranular cytoplasm Blunt cytoplasmic projection C Very immature megakaryocyte ith t o nuclei Minimal amount of finely granular cytoplasm near the nucleus Blunt nongranular pseudopod D Immature megakaryocyte Finely granular cytopla m Multiple nongranular cytoplasmic tags

The shape is usually irregular and there are multiple blunt cytoplasmic projections The nucleus is single relatively large round or slightly indented and has a fine lace like chromatin structure which takes a predominantly acidophilic stain Nucleoli may or may not be present The cytoplasm is relatively small in amount is nongranular takes a basophilic stain and may have a spongy appearance

2 *Immature megakaryocyte* (synonym promegakaryocyte early megakaryocyte

mediate cell in nuclear cytoplasmic and granular structures but differs from it in that granular platelets are demonstrable The platelets usually form small masses a the periphery but the whole cytoplasm may be composed of granular platelet masses The size of the cell varies widely from huge forms that practically fill the oil immersion field to fragments of nuclei with only a few platelets attached to them

5 *Naked nucleus* (Fig 6 L) In this form there is a pyknotic lobulated nucleus and no cytoplasm

There is often asynchronism in the maturation of nucleus and cytoplasm of megakaryocytes Thus one may have a multilobulated nucleus in a cell with basophilic *nongranular cytoplasm or a single round immature nucleus in a relatively small* cell which is actively producing granular platelets Any cell which is producing granular platelets is called mature The hyaline cytoplasmic projections and filaments and the spongy pseudopods are not considered as true platelets but as artefacts due to the tearing of the cell away from its fixed tissue connections or as evidence of amoboid activity Similar platelet like structures are found in reticulum cells malignant cells plasma cells and in the early myeloid erythroid and lymphocytic cells

It is to be noted that the principal criteria for the differentiation of megakaryocytes are the granules[5] [8] and the presence or absence of granular platelets The megakaryoblasts have no granules the immature cells a few fine granules unevenly distributed the intermediate cell has coarse granules fairly evenly distributed but no well defined platelets and the mature cell has coarse granules and platelet formation

One variant of the immature megakaryocyte (fig 5 G is a cell with a perinuclear granular cytoplasm surrounded by a symmetrical ring of coarsely vacuolated cytoplasm and at the extreme periphery cytoplasmic attachments that have a nongranular hyaline or finely spongy character This cell has been interpreted by others to be a degenerate form but we consider it to be a form transitional between the immature and intermediate form The well defined spongioplasm probably represents an area of cytolysis which leads to a shedding of the peripheral cytoplasm and attachments to other cells and to the production of a free granular cell which from that stage on produces true platelets

The Differential Megakaryocyte Count

The differential megakaryocyte counts in our series were made by examining 25 or more megakaryocytes For the control series 50 smears of bone marrow from patients with nonhemorrhagic conditions who had no evidence of blood dyscrasias were examined The distribution of the various stages of the megakaryocyte is given in figure 7

In the control series the mature megakaryocytes which are actively producing platelets are the predominant cells whereas in idiopathic thrombopenic purpura the intermediate cell without platelet production is predominant In idiopathic thrombopenic purpura there is also a relative increase in immature forms and in naked nuclei

3 *Intermediate megakaryocyte* (fig 5 H 6 I J) This cell is quite large often measuring 50 to 80 microns in diameter Its shape is usually round and the margins relatively smooth but the edge may be frayed or there may be cytoplasmic tags

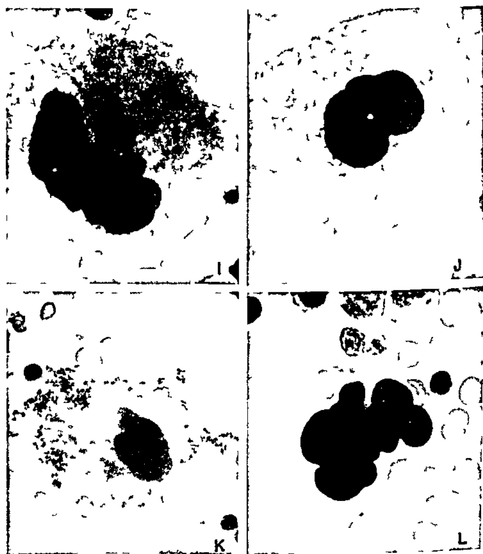


FIG 6 I Intermediate megakaryocyte Large cell Relatively smooth margin Hyperlobulated pyknotic nucleus Fairly evenly distributed coarse granules No platelet differentiation J Intermediate megakaryocyte K Mature megakaryocyte Granular platelet differentiation L Naked multilobulated pyknotic nucleus

The nucleus is relatively small is multilobular and has a coarse chromatin structure The cytoplasm varies in shade from blue to pink and is diffusely granular The granules are relatively coarse There may be vacuoles in the cytoplasm and a tendency toward a clumping of the granules but there is no differentiation of well defined granular platelets

4 *Mature megakaryocyte* (fig 6 K) The mature megakaryocyte resembles the inter

TABLE 4—*The Number and Distribution of Megakaryocytes in Smears of the Marrow Before and After Splenectomy*

Patient		Number per 10,000 nucleated cells	Megakaryoblasts	Immature	Intermediate	Mature	Naked Nuclei
M R	2 days before	48	0	16	76	6	2
	6 days after	6	0	0	20	68	12
K D	2 hours before	17	2	18	54	0	26
	43 days after	5	0	0	30	15	55

TABLE 5—*Prognosis in Relation to the Number of Eosinophils in the Bone Marrow (Idiopathic Hemorrhagic Purpura)*

Eosinophils per 1,000 [illegible]	Deaths	Deaths during recurrence	Improvement with [illegible]	Cure	Period of observation
		With Splenectomy			
					yrs
21				+	2
30				+	1
31	+				
32				+	2
37				+	1
38		+			
39				+	6
39				+	1½
40				+	5
40				+	1
51			+		6
54	+				
60			+		4
61			+		2
71				+	½
77				+	4
80			+		½
107	+				
110			+		2
140			+		1
158	+				
66				+	1½
		Without Splenectomy			
0	+				
31				+	1
32				+	2
39				+	2½
50	+				
69	+				
77				+	5
80				+	½
127			+		2
156				+	6
191	+				

The finding of reduced platelet formation and immaturity of cells is in agreement with the findings of numerous workers and confirms the original concept of Frank[9] and of Minot[10] that there is dysfunction of the megakaryocytes which prevents normal platelet formation

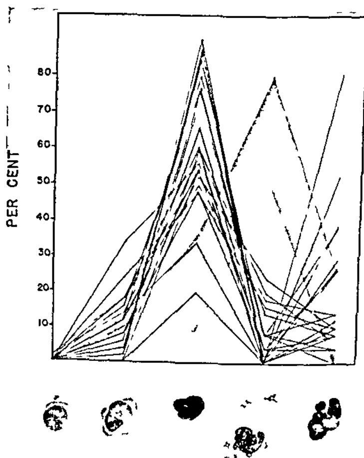


FIG 7 Frequency distribution of megakaryoblasts immature interm diate and mature me gakaryocytes and naked nuclei in sternal bone marrow sm ars of 22 patients with idiopathic hemorrhagi purpura in the acute stage on whom splenectomy was p rform d The rate of variation in nonhemorrhagic conditions is repres nted by th shad d area

THE EFFECT OF SPLENECTOMY ON THE MEGAKARYOCYTES

Sternal marrow aspirations were performed on two of our patients before and after splenectomy (table 4) In both instances there was a decrease in the relative number of megakaryocytes after splenectomy and an increase in the mature platelet producing cells Similar decreases in the number of megakaryocytes have been noted by Wiseman Doan and Wilson and by Limarzi and Schleicher Limarzi and Schleicher Dameshek and Miller and others have also observed that following splenectomy there is a decrease in early forms and an increase in adult forms

In one of our patients the marrow examination after splenectomy during a recurrence of the purpura revealed a megakaryocyte number and differential which was essentially the same as before splenectomy

Table 4.—*The Number and Distribution of Megakaryocytes in Smears of the Marrow Before and After Splenectomy*

Patient		Number per 10,000 nucleated cells	Megakaryoblast	Immature	Intermediate	Mature	Naked Nuclei
M. R.	2 days before	48	0	16	-6	6	2
	6 days after	6	0	0	20	68	12
K. D.	2 hours before	17	2	18	54	0	26
	43 days after	5	0	0	30	15	55

Table 5.—*Progress in Relation to the Number of Eosinophils in the Bone Marrow (Idiopathic Hemorrhagic Purpura)*

Eosinophils per 1,000 mature granulocytes	Deaths	Deaths during recurrence	Improvement with recurrences	Cure	Period of observation
With Splenectomy					
					yrs
21				+	2
30				+	1
31	+				
32				+	2
37				+	1
38		+			
39				+	6
39				+	1½
40				+	5
40				+	1
51			+		6
54	+				
60			+		4
61			+		2
71				+	½
77				+	4
80			+		2
107	+				
110			+		2
140			+		1
158	+				
166				+	1½
Without Splenectomy					
0	+				
31				+	1
32				+	2
39				+	2½
50	+				
69	+				
77				+	5
80				+	1
127			+		2
156				+	6
191	+				

Prognosis Based Upon the Number of Eosinophils in the Marrow

Schwartz[11] in 1945 reported that in thrombopenic purpura increased numbers of eosinophils in the marrow signify a favorable response for spontaneous recovery while scant numbers foretell a chronic course and necessity for splenectomy He made direct smears of material aspirated from the sternum The number of eosinophils per 1000 granulocytes at the metamyelocyte stage or older were counted

Schwartz interprets an increase in the eosinophils of the marrow as a manifestation of an allergic state or evidence of sensitivity to infections drugs or allergenic foods He states that those with more than 50 eosinophils per 1000 granulocytes have an acute onset a relatively benign course and spontaneous and complete clinical and hematologic recovery and that removal of the spleen in such allergic thrombopenias is not necessary

The relation of the eosinophils to prognosis was studied in our series using the same method of counting eosinophils as recommended by Schwartz No correlation was found between the eosinophil counts and the deaths recurrences or cures with or without splenectomy (table 5) We do not accept the thesis that eosinophil increase in the bone marrow is always indicative of sensitivity to exogenous food, drug or other factors There is evidence that the eosinophilia may be a reaction which is secondary to the hemorrhage into the skin or other tissues [1]

Discussion

The variation in the number of megakaryocytes in different individuals with idiopathic thrombopenic purpura is in part due to true differences in individuals and in the distribution of bone marrow giant cells as has been shown in biopsy material by Lawrence and Knutti[13] and in autopsy material by others but much of the variation is due to dilution of the marrow cells with peripheral blood and other technical factors Megakaryocytes and particularly early forms are partially fixed cells which are not readily aspirated These cells are fragile and easily destroyed by any manipulative procedure They contain large amounts of thromboplastin and tend to get caught in fibrin webs which rapidly form around them They are large cells which are pushed toward the margins and ends of slide preparations We have found that the megakaryocytes in the best of coverslip preparations were unevenly distributed and that counts made on the same smears by the same or different individuals varies as much as 100 per cent

Since there are unavoidable errors involved in megakaryocyte counting a wide range of variation in different individuals and within the range of observed values no correlation between the megakaryocyte counts and prognosis it is obvious that there is little use in undertaking the laborious task of making actual counts

Prognosis and indications for splenectomy are determined not from the megakaryocyte study alone but from the study of the entire patient If the diagnosis is aplastic anemia leukemia or secondary thrombopenia splenectomy is not indicated

If in 2 drops of material aspirated from the marrow (4 coverslip or two slide preparations) there are twenty or more megakaryocytes if the majority of the bone marrow giant cells are immature or intermediate and not actively producing

platelets and if the rest of the marrow peripheral blood and clinical picture fits the diagnosis of idiopathic thrombocytopenic purpura is made and splenectomy may be recommended

The finding of numerous megakaryocytes which are actively producing granular platelets is against the diagnosis of essential thrombopenic purpura or is indicative of a spontaneous remission in a known case Splenectomy in such cases is contra indicated

The peculiar distribution of megakaryocyte types in some patients with essential thrombopenic purpura (fig 7) in which there are few mature forms yet an increased number of naked nuclei suggests that the intermediate cells disintegrate without going through the platelet producing stage This is additional evidence that the low platelet count found in this disease is due to defective formation of platelets rather than to excessive destruction outside of the bone marrow

Summary and Conclusions

1 Observations made on the bone marrow smears of 36 patients with idiopathic hemorrhagic purpura and the correlation of the findings with the clinical picture are presented The control series consisted of 50 patients with nonhemorrhagic conditions without blood dyscrasias

2 The bone marrow in idiopathic hemorrhagic purpura is hyperplastic There is a slight myeloid and erythroid immaturity and in some cases a slight eosinophilia and lymphocytosis

3 The megakaryocyte counts ranged from 3 to 59 per 10 000 nucleated cells with an average of 17 In the control series the megakaryocytes ranged from 1 to 54 per 10 000 nucleated cells with an average of 16

4 There appears to be no correlation between the number of megakaryocytes found in the marrow smears during the acute phase of the disease and the prognosis with or without splenectomy There is also no apparent correlation between the number of megakaryocytes and the platelet response following splenectomy

5 The megakaryocytes were classified as megakaryoblasts immature intermedi ate or mature megakaryocytes or as naked nuclei The principal criteria for the differentiation of megakaryocytes are the granules and the presence or absence of granular platelets

6 The differential megakaryocyte counts were made by examining 25 or more megakaryocytes In the control series the mature megakaryocytes which are ac tively producing platelets are the predominant cells whereas in idiopathic hemor rhagic purpura the intermediate cell without platelet production is predominant

7 Marrow studies on 2 of our patients before and after splenectomy revealed a decrease in the relative number of megakaryocytes and an increase in the number of platelet producing cells following operation

8 No correlation was found between the eosinophil counts and the deaths re currences or cures with or without splenectomy

9 The principal value of the bone marrow examination in cases of suspected idiopathic hemorrhagic purpura is to exclude leukemia and aplastic anemia

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THROMBOCYTOPENIC PURPURA COMPLICATING GOLD THERAPY FOR RHEUMATOID ARTHRITIS

Report of Three Cases with Spontaneous Recovery and One Case with Recovery Following Splenectomy

By Stacy R Mettier M D Alice McBride A B and Jonah Li M D

IT IS A WELL established fact that certain drugs may cause thrombocytopenic purpura With the introduction of the ever increasing number of new drugs in the treatment of disease this untoward reaction becomes more and more a medical problem Mild to severe hemorrhagic tendency has been reported as occurring during the course of administration of sedormid the sulfonamids the arsphenamines sodium salicylate and other drugs Recently an increasing number of occurrences of thrombocytopenia have been reported as resulting from the injection of gold salts for rheumatoid arthritis

Hartfall Garland and Goldie[1] treated 900 patients with arthritis with injections of gold salts and among this group there were 11 cases of purpura hemorrhagica Three of these progressed to a fatal termination There was no correlation between the severity of the platelet reduction and recovery or fatal termination

Of the 245 patients treated with gold salts by Cecil Kammerer and De Drume 3 showed purpuric lesions in the skin The lesions subsided when the drug was discontinued

Price and Leichtenstritt[3] reported a series of 100 patients who had received gold salts and 3 developed thrombocytopenia Two of the patients recovered after withdrawal of the drug but the third died

Short Beckman and Bauer[4] treated a group of 47 patients with rheumatoid arthritis with injections of myochrysine in doses of 100 milligrams of the drug One of their patients developed a severe hemorrhagic tendency with bleeding from the gums and nose Platelets were not found present in the blood smear In spite of repeated transfusions the patient continued to show widespread bleeding and death occurred five weeks after the onset of the purpura

During the past seven years in the wards and out patient department of the University of California Hospital 160 patients on whom a diagnosis of rheumatoid arthritis had been made were given gold salt therapy During the course of treatment 4 of the patients developed thrombocytopenia and purpuric manifestations The results of the observations made on these 4 patients are reported

Case 1

A W A white American female 44 was examined May 28 1943 She stated that three years earlier she had first noticed a painful swelling of the middle joint of the third finger of the right hand Since that time swelling accompanied by pain had appeared in other joints of the fingers and spread to involve the

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S pported by a grant from the Cecilia Benfield Fund and the James Foundation

wrists shoulders elbows and ankles In recent months she had lost considerable weight but was unable to estimate the amount

Physical examination The patient appeared of small stature and structure A survey of the skeletal system revealed a moderate degree of swelling of the interphalangeal joints of both hands There was a soft fluctuant swelling over the dorsum of the right wrist Both ankles were swollen and markedly tender to the pressure of the fingers

Laboratory data Examination of the blood showed hemoglobin 64 per cent (11 grams with standard at 17 2 grams per 100 cc) erythrocytes 3 780 000 per cubic millimeter leukocytes 8800 per cubic millimeter and platelets 300 000 per cubic millimeter Sedimentation of the erythrocytes was 37 millimeters in one hour The plasma content of ascorbic acid was 1 7 milligrams per 100 cc and the uric acid was 3 3 milligrams per 100 cc

Diagnosis Rheumatoid arthritis hypochromic anemia

Course of the illness The patient was returned to her physician for treatment with the advice that she receive injections of myochrysine in doses of 50 milligrams intramuscularly at intervals of one week She was examined again December 15 1943 at which time there had been a gain in weight of 6 pounds She considered herself to be in good health and there was no apparent swelling of the joints previously involved The hemoglobin was 116 per cent (Sahli) and the rate of sedimentation of the erythrocytes was 2 millimeters in one hour The gold therapy was discontinued

Three month later March 11 1944 the patient was admitted to the hospital because of recurrence of swelling of the joints and a moderate hypochromic anemia Daily physical therapy consisting of radiant heat massage and passive exercise to the involved joints was given the patient One month later April 17 the patient received an injection of myochrysine 25 milligrams in the buttocks The following day a fine purpuric rash appeared and was widely spread over the body Soon after there was moderate epistaxis on two occasions

Examination of the blood showed erythrocytes 3 050 000 per cubic millimeter hemoglobin 59 per cent (8 5 grams) Sahli The white blood cells were 6650 per cubic millimeter It was of interest that 3 per cent of the total leukocyte count was of the eosinophile variety The blood platelets were 90 000 per cubic millimeter and there was a markedly increased fragility of the blood capillaries

The patient was given a whole blood transfusion of 500 cubic centimeters One week after its appearance the purpura had completely disappeared and the platelets had returned to normal numbers

Case 2

J W A white American man 50 reported to the outpatient department of the University of California Hospital March 8 1946 complaining of pain in the shoulders elbows wrists hands knees and feet extending back over a period of ten years Although he had carried on his usual occupation as a clerk he had found it more and more difficult to get about because of progressive disability of the lower extremities and limited motion of the elbows and shoulders Surgical removal of the thyroid gland had been accomplished at the age of 18 in an attempt to relieve hyperthyroidism complicated by exophthalmus

Physical examination The arms were held rigidly to the patient s sides With passive motion practically no abduction of the right arm was possible but elevation of the left arm to 20 degrees was accomplished with difficulty There was mild slightly painful swelling of the various interphalangeal joints and there was thickening of the tissues on the dorsum of the wrists and about the knees and ankles There was considerable atrophy of the muscles adjacent to the involved joints An exophthalmus was still present as a residuum of the previously overactive and enlarged thyroid gland

Laboratory data The blood count showed hemoglobin 100 per cent (14 5 grams) Sahli erythrocytes 4 990 000 per cubic millimeter and leukocyte 9960 per cubic millimeter The platelets were reported to appear in an abundance on the stained films of blood The blood uric acid was 3 5 milligrams per 100 cc Sedimentation of the erythrocytes occurred at the rate of 33 millimeters in one hour An estimation of the rate of basal metabolism was recorded at 8 per cent above normal The plasma cholesterol was 300 milligrams per 100 cc

Course of the illness On April 1 1946 the patient was given his first injection of 50 milligrams of myochrysine (gold sodium thiomalate) The injections were repeated at intervals of one week accompanied by considerable improvement in the patient s well being On December 17 1946 the patient received his

twenty fourth injection which on this date made a total of 1200 milligrams of the colloidal suspension (myochrysine)

The patient failed to report for further treatment until seven weeks later February 4 194 when gold therapy was resumed He received the usual injection of 50 milligrams of myochrysine The patient re turned on February 10 stating that two days before minute areas of hemorrhage had suddenly appeared in the skin of the lo er extremities and forearms follo ed some hours later by hemorrhagic blebs in the mucous membranes of the lips That e ening he passed a very loose stool that contained a considerable amount of bright red blood The patient also observed oozing of blood from the gingival border of the gums

Laboratory data Examination of the blood on February 10 showed hemoglobin 90 per cent erythrocytes 4 000 000 per cubic millimeter and the leukocytes 7500 per cubic millimeter The blood platelets were estimated at 80 000 per cubic millimeter A specimen of blood clotted at the end of eight minutes but failed to show retraction after several hours The prothrombin content of the blood was 90 per cent of normal Sternal puncture ho ed an essentially normal marrow picture with single cells and scattered islands of nucleated red cells and granulocytes The megakaryocytes were increased in number but showed a greatly diminished platelet production

Further injections of colloidal gold were discontinued and the patient was requested to report at fre quent intervals for observation of the hemorrhagic tendency On examination February 13 the hemor rhagic lesions had faded considerably The platelets were 145 000 per cubic millimeter The patient re ported again February 17 when all evidence of hemorrhage had subsided and the platelets were 1 0 000 per cubic millimeter

Case 3

S G A white American female 33 as examined Nov 5 1946 Her illness started six years prior to entry when she noted pain low in the back and the midthoracic region Relief was obta ned following the application of heat and the use of mild analgesics S nce then there had been a recurrence of symptoms at intervals of one to two years One year ago she became aware of pain in the cervical region and the gradual development of pain and swelling of the small joints of the hands These symptoms ha e per isted since

Physical examination The patient appeared slightly underweight and e idenced discomfort along the back There was sl ght swelling of the first second and third metacarpophalangeal joints of both hands and of the proximal phalangeal joints of the middle fingers Pain was elicited in those joints on pressure There was stiffness of the cervical spine and forward flexion was limited to one fourth the normal range There was spasm of the paravertebral muscles in the lumbar region Forward flexion of the l mbar spine was limited to three fourths of the expected normal range During this maneuver flattening of the spine became apparent in this region No abnormalities were found on examination of the heart and lungs Neither the spleen nor the liver could be felt

Laboratory data The blood count showed hemoglobin 69 per cent (11 9 grams) erythrocytes 3 820 000 per cubic millimeter and leukocytes 6800 per cu mm The platelets were 350 000 per cubic millimeter The plasma ascorbic acid was 1 9 mg per 100 cc The rate of sedimentation of the erythrocytes was 26 millimeters per hour On examination of the roentgen films narrowing of the cervical-dorsal interspace was apparent and the margins were sclerotic The regional apophyseal jo nts especially the eleventh do sal showed marginal sclerosis

D ag osis Rheumatoid arthritis of the spine and of the metacarpophalangeal joints

Course of the illness Over a period of seven days the patient was given a course of roentgen irradiation to the spine consisting of a total of 675 R Four weeks later a similar course of treatment was pursued Soon after this was followed by complete relief from the back pain

On Nov 14 1946 one week after the patient had been l st e posed to the roentgen rays she was given intramuscularly an injection of myochrysine 0 025 grams (gold sodium th omalate) Three weeks later when the patient reported for the expected third injection she stated that for the past t o days a fine red nonpruritic rash h d been visibl over the lower extremities There was no bleeding from the gums

Labor tory data The blood count on Nov 27 showed hemoglobin 81 per cent (11 68 grams) Sahli erythrocytes 3 930 000 per cu mm and leukocytes 3250 per cu mm The blood platelets were greatly

reduced and showed 65 000 per cu mm on actual count A specimen of blood failed to show clot retraction When the Dalldorf apparatus was reduced to a pressure of minus 20 millimeters of mercury and applied to the skin of the arm above the antecubital fossa it induced the appearance of large numbers of petechial hemorrhages This indicated increased fragility of the capillaries A sternal puncture showed an essentially normal marrow with the exception of a slightly increased number of megakaryocytes These failed to show evidence of platelet production

Further injections of gold compound were discouraged One week later on examination the petechial rash had almost entirely faded and there was no evidence of new lesions When the platelets were enumerated there were 160 000 per cu mm and four weeks later 200 000 per cu mm

Comment

The three case histories reported above are examples of thrombocytopenia arising secondary to intramuscular injections of a colloidal suspension of gold (myochrysine) This was accompanied by mild purpuric manifestations in two of the patients and was limited to petechial hemorrhages in the skin In the third patient the hemorrhages were not only more extensively distributed over the body but were accompanied by bleeding from the nose and gastrointestinal tract After three or four days without specific therapy the blood platelets appeared spontaneously in the circulating blood in all of the patients in increasing numbers coincident with the disappearance of the hemorrhagic tendency At no time did the lives of the patients appear to be in jeopardy

Case 4

R B a white American female 44 was first examined Nov 15 1945 She stated that one year earlier she noted swelling of the small joints of both hands Soon after swelling accompanied by pain appeared in the wrists elbows knees ankles and small joints of the feet During the past four months there had been a loss of 30 pounds in body weight

Physical examination On admission she appeared ill and underweight Locomotion was accomplished with considerable pain and difficulty There was marked pallor of the mucous membranes and of the palms of the hands There was swelling of the middle interphalangeal joints of both hands of the metacarpophalangeal joints and at the wrists There was slight ulnar deviation of the fingers Extension of the forearms on the elbows was limited to 165 degrees The shoulder joints were restricted to less than one half their normal range of motion There was slight degree of swelling of the left knee and both ankles A low pitched murmur was heard over the apex of the heart during systole and was thought to be of hemic or gin The spleen and liver could not be palpated

Laboratory data The blood count showed erythrocytes 3 060 000 per cubic millimeter hemoglobin 38 per cent (6 5 grams) enco Sheard Sanford electric photometer calibrated to 17 2 grams of hemoglobin per 100 cc blood and leukocytes 7100 per cubic millimeter The differential count was within normal range The content of whole blood uric acid was 4 2 mg per 100 cc and the plasma ascorbic acid was 0 45 mg per 100 cc The hematocrit was 22 5 per 100 cc of blood The sedimentation rate (corrected) was 10 mm per hour The blood platelets were 220 000 per cu mm The mean corpuscular volume was 70 cubic microns the mean corpuscular hemoglobin concentration was 28 per cent the mean corpuscular hemoglobin was 18 micrograms

Clinical diagnosis Rheumatoid arthritis hypochromic anemia

Course of the illness On Nov 20 and 23 the patient received transfusions of 500 cc each of whole blood which were followed by an increase in the hemoglobin to 60 per cent (10 3 grams) and in the red cell count to 3 74 M Medication consisted of the oral administration of ascorbic acid 100 mg and ferrous sulphate 1 2 grams in divided doses daily On Nov 19 the patient received her first injection of colloidal gold (myochrysine gold sodium thiomalate) 0 025 grams or 0 0125 grams actual gold At the end of the fourth week the patient complained of a generalized itching rash which was erythematous in character The injections of colloidal gold were discontinued Two weeks later Dec 31 the patient returned stating

that the rash had disappeared She stated that she was having much less pain was more active and her general feeling of well being had improved There was an increase in weight from 105 pounds at the onset of illness to 111 pounds on this date

On Jan 21 1946 the date she should have received her ninth injection of colloidal gold there suddenly appeared some oozing of blood about the lower right premolar and there were innumerable petechial hemorrhages on the lower extremities The blood count showed erythrocytes 4 810 000 per cu mm hemoglobin 73 per cent (10 5 grams) Sahli leukocytes 11 100 cells per cubic millimeter and platelets 215 000 per cubic millimeter Following application of the sphygmomanometer cuff to the arm and elevating the pressure to just above diastolic there was the appearance of petechial hemorrhages in the antecubital fossa Chrysotherapy was discontinued until on examination March 18 1946 the hemorrhagic tendency had completely subsided On this date the hemoglobin had increased to 84 per cent but there were no significant changes in the erythrocytes of platelets

Injections of colloidal gold were started and repeated at intervals of one week until July 8 1946 when petechial hemorrhages reappeared in the skin On this occasion the blood platelets were found reduced to 100 000 per cubic mm The patient reported at intervals of one week until Sept 16 when she stated that she had had a severe epistaxis and for ten days a constant menstrual flow Examination of the blood showed erythrocytes 3 670 000 per cu mm hemoglobin 55 per cent platelets 110 000 per cu mm and leukocytes 450 per cu mm There were 5 per cent eosinophiles

During the month of September the patient suffered frequent attacks of epistaxis and on the occurrence of the menstrual flow the period extended over fourteen days instead of the usual four days On two successive days she was given whole blood transfusions of 500 cc Roentgen irradiation of the ovaries was resorted to as an attempt to induce artificial menopause The menses failed to appear on the expected date but bleeding elsewhere had become more profuse On Oct 23 there was continuous oozing of blood from the nose and the gingival margins Large ecchymoses appeared in the skin and large numbers of red blood cells were observed in the urine An enumeration of the platelets revealed 10 000 per cu mm of blood The bleeding time was greatly prolonged A specimen of blood showed a tendency to coagulate after a lapse of ten minutes but the clot was soft friable and failed to retract A sternal puncture showed marked erythropoietic activity as evidenced by large islands of nucleated normoblasts and erythroblasts There was moderate myeloid hyperplasia There was a slight increase in the number of megakaryocytes These seemed rounded somewhat opaque and showed no evidence of platelet production For the past week the patient had been given four transfusions of whole blood without materially altering the bleeding It was apparent that the patient s condition was becoming critical and was approaching the state of purpura fulminans On Oct 24 splenectomy was performed by Dr Leon Goldman The spleen was found enlarged to about twice its normal size With the exception of a mild febrile reaction following a transfusion the patient s postoperative course was without untoward event The abnormal bleeding stopped Four hours after the operation was terminated the blood platelets were 210 000 per cu mm twenty four hours later they were 325 000 per cu mm and three months later were 550 000 per cu mm

Comment

The fourth case history reported here differed essentially from the preceding three in the duration and severity of the hemorrhagic tendency Two months after the first episode of thrombocytopenia a second series of injections of gold salt was started only to be interrupted at the end of four months because of a recurrence of bleeding For the next three months the patient continued to exhibit spontaneous bleeding of varying degree Finally with the occurrence of frank hemorrhage from the mucous membranes it was apparent that there was an acceleration in the bleeding disturbance Splenectomy was performed following which there was a complete disappearance of the abnormal tendency to bleed

Discussion

The cause for the thrombocytopenia which may occur during the course of gold therapy is not well understood It is possible that it may be an allergic phenomenon

wherein the megakaryocytes become so altered as to interfere with the production of platelets In the three patients on whom sternal puncture was performed it was observed that the megakaryocytes although slightly increased in number appeared to be deficient in platelet production This reaction however is not unique for the thrombocytopenia that occurs in gold therapy since various investigators including Frank[5] Limarzi[6], and Dameshek and Miller[7] have described the apparent inactivity of the megakaryocytes in cases of socalled idiopathic thrombocytopenia

In recent times the term hypersplenism has appeared in the medical literature It is presumed that an abnormal spleen acts upon the bone marrow or more specifically the megakaryocytes to produce an inhibitory mechanism Emphasis for this postulation is found in the greatly increased production of platelets soon after splenectomy

The management of a patient with thrombocytopenia induced by gold salt therapy offers a serious problem to the clinician In most instances the abnormal bleeding is of short duration On the other hand the bleeding may become enhanced and prolonged may fail to respond to transfusions and ultimately lead to the patient s death The question arises as to whether splenectomy should or should not be performed It has been the consensus of opinion among surgeons[8] that splenectomy is not indicated but rather contraindicated in secondary thrombocytopenic purpura due to severe infections or intoxications In none of the other reported cases of thrombocytopenia secondary to gold had splenectomy been attempted In the present case the mild hemorrhagic disturbance of four months duration became suddenly accelerated by the appearance of marked bleeding from the mucous membranes It was felt that since the life of the patient was in danger splenectomy was advisable Soon after the operation there was a dramatic response of the platelets which led to the recovery of the patient

Of interest in connection with the case reported here is the one reported by Farfel [9] wherein a patient with thrombocytopenia due to sulfathiozole therapy recovered following splenectomy

Summary

Of 160 patients treated with gold salt therapy four developed thrombocytopenia with mild purpuric manifestations In three patients there was a spontaneous remission and the hemorrhagic tendency disappeared in about one week s time

In the fourth patient there was a second occurrence of thrombocytopenia which persisted over a period of four months and finally failed to respond to transfusions Splenectomy was followed by a dramatic rise in the blood platelets and recovery of the patient

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INCONSTANCY AND VARIABILITY OF THE VASCULAR FRAGILITY TEST EVEN IN PURPURIC CONDITIONS

By J Roskam, M D Ch Renard M D and L Swalue M D

THE PATHOGENESIS of purpura is mysterious and complex This is true for the origin of uncontrollable hemorrhages in Werlhof s disease and similar conditions Clinical and experimental observations have indeed proved that the very long bleeding times occurring in hemogenic and hemophilo hemogenic syn dromes are due to the association of a hemic and a vascular factor (Roskam [11-13]) Although the existence of the latter factor is established beyond doubt its nature is not yet completely elucidated

The breaking out of intradermal purpura seems also to result frequently from multiple factors (Grenet[4] Bedson[1]) seldom analyzed as yet

Several authors have however emphasized the importance in the production of petechiae of the fragility of vessels of purpuric patients as namely evidenced by the ease with which venous stasis produced a petechial eruption in these patients

This induced purpura was described in 1911 by Frugoni and Guigni later by Weill (of Lyon) and Chalier[16] in certain hemorrhagiparous conditions The same year Leede[5] erroneously considered it as pathognomonic of scarlet fever Later this phenomenon was systematically studied and was found by many authors in various conditions intoxications avitaminoses diseases of the endocrine organs of the spleen the reticulo endothelial system of the sympathetic nervous system (Stephan[15]) in hypertension solitary or combined with arteriosclerosis in neph ritis in endocarditis lenta (Weissman[17]) in diabetes in rheumatic patients given large amounts of sodium salicylate in patients with gastro duodenal ulcers treated with a milk diet and alkaline powders in patients with chronic glaucoma (Roskam[9]) in erythremia icterus certain forms of tuberculosis (Schour[14]) etc

The intensity of the purpura induced by venous stasis signe du lacet capillary fragility test capillary resistance test or tourniquet test etc is approximated by the number of petechiae which appear during the test

One of us has however repeatedly pointed out the qualitative and not quantitative aspect of the eruption i e the importance of the size of the differ ent purpuric elements Petechiae with a diameter above one millimeter possess a significance similar to that of increased bleeding time (Roskam[10])

These proportionally large petechiae are observed in subjects with a hemorrhagic condition The more severe the condition the larger is generally the diameter not of all petechiae but of a certain number of them

After this brief review we will take the opportunity of describing two recent clinical observations to underline the diverse results in different circumstances of the vascular fragility test as we prefer to call the capillary fragility test for no one has proved that only capillaries are involved in that test

From the Institute of Medical Clinic and Pathology University of Liege Belgium

Case Histories

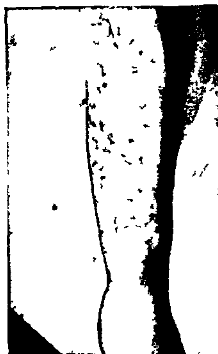
Case 1 V Michel male 16 high school student

Fam ly history irrelevant

Personal history measles scarlet fever diphtheria appendectomy in childhood

Present disease At the beginning of December 1946 appearance of an eruption not disappearing by itropression made up of small elements grouped in clusters on the antero-medial aspect of the fore arms and thighs Later exten ion of this eruption mainly to the shoulders to the anterior side of the legs and to the ankles Purple at their appearance the small erupti e elements turn later bro n pink and yellow before disappearing Several eruptive wa es follow always occurring in the skin areas involved in the first attack

Phys cal examinati n On Jan 10 194 nothing unusual except for pigmented sequelae of previous eruptions and for a few small submaxillary and axillary nodes



F G 1

S d m tation rate 3 mm in the first hour (Westerg en)

Hem gl b n 55 per cent

R B C 4 200 000 *Morphology* normal

W B C 200

D fferential Neutrophilic polymorphonuclea s 48 pe cent eo inophilic polymorphonuclears 3 per cent lymphocytes 43 5 per cent monocytes 5 5 per cent

Plat lets 220 000 *Morphol gy* normal

Bleed ng time Right ear 1 30 1 1 3 30 2 Left ea 1 30 1 30 - 30 1 30

Cl tt g t me 22 22 22 22 (no mal 16 to 24)

Pr thromb n time (Qu ck) 100 per cent

Bordet Wasserman Me nicke Paul and Bunnel *react ons* negative

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Fig 3



Fig 4

*Vascular fragility test** quantitative ++++ qualitative +++ only in the cutaneous areas corresponding to previous eruptions as the petechiae induced by venous stasis are grouped in clusters and form eruptive blotches separated by areas of normal or almost normal skin (figs 1 2 and 3)

One week later despite the daily intake of 40 mg of citrin similar vascular fragility test quantitative ++++ qualitative +++ in the cutaneous areas with sequelae of previous eruptions almost negative between these areas

Case 2 Jacques male 17 high school student

Family history irrelevant

Personal history measles chicken pox mumps whooping cough in childhood appendectomy in 194- frequent bronchitis gastro intestinal upset with fever in October 1946



FIG 2

Present disease On Saturday January 25 1947 the patient ate lobster with cocktail sauce duck and pate de foie gras Normal activity from January 26 to 28 but exposure to very cold weather du ing these three days On Tuesday 28 purpuric stripes (length 1 – cm width ½–2 cm) suddenly appeared on the face and neck On the 29th fever (about 38 C) which lasted for two days headache and nausea whereas the eruption faded out progressively and disappeared on Feb to

On Feb 2 at 8 P M intake of t o boiled eggs and again exposure to severe cold At 9 P M as his

* The vascular fragility test which we used is the test described by one of us in 1929 under the name of signe due brassard application above the elbo of the cuff (brassard) of Boulitte s oscillometer inflated half way between the maximal and minimal arterial pressure of the patient The pressure is held for 15 minutes After decompression there is examination of the induced purpura on the whole surface of the forearm and hand and not as later proposed by Wright and Lilienfeld [18] in a small area of the supero-medial aspect of the forearm

As the history and tests suggested an anaphylactic purpura preparations of egg white mayonnaise foie gras and lobster were scratched into the skin no reaction Similarly intradermal reactions with milk tuberculin Dmelcos antistaphylococcic vaccine remained negative as well as attempts to find a possible fecal infection The intake of foie gras on Feb 20 did not bring any of the reactions characteristic of Widal s digestive hemoclasia nor any rash

However the patient—who had been kept at normal temperature—ate on Feb 6 at noon lobster cocktail sauce and in the evening some foie gras On the next morning the purpuric stripes appeared at the base of the neck and on the anterior aspect of the forearms the latter spots were surrounded by an area of congestion without pruritus The eruption induced on February 6 and 7 was much milder than the previous ones This was the last of the purpura of this patient

On Feb 14 Paul and Bunnel s *reaction* 1/16 +++ 1/32 ++ 1/64 +

Comments

Both cases reported had a purpuric eruption

In the first one the eruption was symmetrical and made up of innumerable petechiae grouped in cluster in maplike areas 0 5 to 2 cm in diameter separated by areas of almost normal skin This relapsing purpura simplex had a protracted course or at least a subacute one The etiology remained mysterious as well as the cause of the different attacks However outside any purpuric attack the vascular fragility test was twice strongly positive quantitatively and qualitatively but only in those cutaneous areas where the previous eruptions had spontaneously occurred

In the second patient the eruption also symmetrical was formed by rather homogenous hemorrhagic streaks not resulting from the coalescence of smaller petechial elements The only real petechiae were observed on the soft palate The etiology of the syndrome probably was alimentary However it is noteworthy that the cuti reactions with the suspicious foods remained negative as well as Widal s tests of digestive hemoclasia and that the purpura simplex of this patient occurred at the same time as an attack of infectious mononucleosis The vascular fragility test was completely negative at the place of the purpuric spots and outside them

Thus in one of the two reported cases of purpura simplex a strongly positive quantitative and qualitative vascular fragility test was present

In the other the vascular fragility test was completely negative

In order to explain the different behavior of the vessels of the two patients during venous stasis one might consider the different nature of the two cases of purpura as also evidenced by differences in the clinical course and in the purpuric eruption

This simple interpretation is probably accurate

Nevertheless a case of constitutional athrombopenic hemorrhagic purpura published by one of us in 1929 (Roskam[8]) might be taken as an argument against it

For this patient we noted on April 5 1927 at the time of his admission to the hospital

Tourniquet test after 15 minutes of compression at 70 mms of mercury with the blood pressure machine there appeared below the right elbow several large petechiae measuring 1–3 mms About 50 were seen on the anterior aspect 40 on the posterior aspect of the forearm Under the same conditions the test being repeated twice no purpuric elements were seen over the left forearm On April 29 we noted

father was weighing and measuring the patient he saw the appearance of a new eruption which reached its maximum in a quarter of an hour More numerous the purpuric spots were localized on the face neck upper part of the chest forearms and wrists Soon afterwards fever (38 2 C) headache and nausea

Physical examination On Feb 3 1947 in addition to the cutaneous eruption (figs 4 and 5) there were slight fever (37 5–38 5 C) which disappeared progressively in ten days a few petechiae on the soft palate a few small nodes in the neck groins and axillae and a palpable spleen reaching the costal margin on percussion

Sedimentation rate 4 mm in the first hour (Westergren)

Hemoglobin 100 per cent

R B C 4 750 000 *Morphology* normal

W B C 6 000

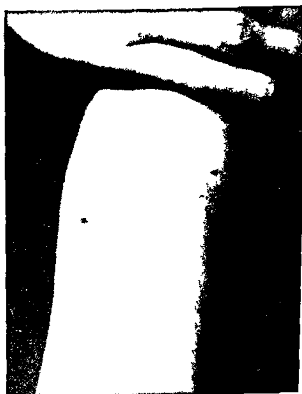


Fig 5

Differential Neutrophili polymorphonuclear 60 eo inophilic polymorphonuclear 2 lymphocytes 31 monocytes 7

Platelets 225 100 *Morphol gy* normal

Bleeding ti ne Right ear 1 1 30 2 3 Left ear 3 2 1 1 2

Clotting tim 21 23 21 23 (normal 16 to 24)

Prothrombin tim (Quick) 100 per cent

Bordet Was erman Meinicke Widal Wright s *reactions* neg tive

Paul and Bunnel s *test* + at 1/8

Hemo ulture negative

Takata Dohmoto s *f c lat fig re* 59 (lo v but still no mal)

Weltman s *coagulation band* 0 4 (no mal)

Vascula fragility test quantitati e nd qualit ti e p act cally negati e on this day as well as later when the eruption was fading or had disappea ed

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Tourniquet test after 15 minutes of compression of the arm at 80 mms of mercury, identical numbers of petechiae appeared over the right forearm as had been noted on April 5 The left forearm at this time showed a very marked purpuric eruption with enumerable punctate petechiae 195 petechiae were present on the anterior aspect of the left forearm together with 6 ecchymoses of about 5 mms in diameter Posteriorly 175 petechiae of about 1 mm in diameter were seen Tourniquet test after 15 minutes of compression at a pressure of 100 cu mms of mercury an intense purpuric eruption about the same both in the right and left arms occurred Numerous petechiae were seen These extended over the dorsum of the hands and over the fingers

We have thus observed a case of constitutional athrombopenic hemorrhagic purpura with during an acute period a vascular fragility test quantitative + qualitative ++ on the right side completely negative on the left side a few days later quantitative + qualitative ++ on the right side quantitative and qualitative ++++ on the left Two years later without hemorrhagic episode the vascular fragility test was strongly and equally positive on both sides

This observation indicates the great variability of the vascular fragility test at different times, and also at different sites in symmetrical areas of the skin in a case of chronic hemorrhagic purpura

Together with the two new cases reported in this paper, it shows the complexity of the factors producing the purpuric eruption The appearance of cutaneous hemorrhages is in no way a simple phenomenon and its mechanism is still unknown The observations of Bedson has shown that the experimental induction of petechiae and hemorrhages sometimes requires the cooperation of a hemic and a vascular factor Reilly Rivallier Compagnon Laplane and Du Buit [6] later confirmed by Frumusan [3] have demonstrated the role of the autonomous nervous system in animals in the production of some hemorrhagic lesions of the gastrointestinal tract These very interesting experiments do not however afford a satisfactory explanation of the clinical observations concerning the apparition of purpura

We hope that this paper will draw attention to this important problem and that it will make experimenters and clinicians conscious of the inconstancy and variability of the vascular fragility test

Summary

Two unusual cases of purpura simplex raise the problem as yet unsolved of the pathogenesis of the purpuric eruption Together with a previous observation of hemorrhagic purpura made by Roskam they show the inconstancy and variability of the vascular fragility test

Acknowledgment

This paper was translated by Raymond G Gottschalk M D whose cooperation is gratefully acknowledged

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countries For a detailed and complete review of this subject Nygaard's monograph[10] should be consulted

Interpretation of the coagulation time All the common procedures for determining the coagulation time are based on timing the interval between the removal of the blood from the vessel and the formation of sufficient fibrin to meet an arbitrarily designed end point The objective of every test is to measure the intrinsic coagulative power of the blood Obviously therefore trustworthy results can be obtained only by performing the test under constant and rigidly controlled conditions and by excluding all outside agents that influence the coagulation reaction Of the latter tissue juice is by far the most important since it contains thromboplastin It is therefore of utmost importance to exclude all traces from the specimen of blood used for determining the clotting time Obviously blood obtained by skin puncture (capillary blood) is utterly unsuitable since not only does it contain an appreciable amount of tissue fluid but even more serious the amount cannot be controlled Thus Christie[11] demonstrated that there were variations in the clotting time of the different drops of blood collected from the same puncture and Lee and White[3] cite the example of a hemophilic blood which had a coagulation time of 50 minutes for venous blood and only 5 minutes for capillary blood It must be emphasized that the coagulation time of capillary blood irrespective of the method used is worthless and unreliable for clinical purposes Even in taking blood by veni puncture enough tissue thromboplastin may occasionally gain entrance to reduce significantly the coagulation time as Jaques and his co workers[1] have recently demonstrated It is not unusual to obtain a coagulation time of 8 to 10 minutes in a hemophilic subject whose true coagulation time is 1 hour merely by causing slight trauma in drawing the blood

Standardization of the coagulation time There is a distinct need for a simple and uniform procedure In the United States the Lee White test is gradually replacing the other methods This procedure is simple and yields as much and as accurate information as any test of coagulation so far devised but unfortunately the test has not been rigidly standardized and at present there is no strict uniformity in the details of the procedure

In order to devise a standard procedure it is necessary to consider the more important factors that influence the coagulation of blood in a test tube They are (1) temperature (2) size of tube and (3) the nature of the surface of the tube

Temperature Since the coagulation of blood involves chemical and enzymatic reactions it is obvious that the temperature must significantly influence the speed of the process This is clearly demonstrated by the results in table 1 in which the clotting times obtained at 22 C and 37 C are recorded The marked effect on hemophilic blood is particularly noteworthy Similar observations have been recorded by Parek and Stetson[8] The need for adopting a constant temperature at which the determination is carried out is evident Body temperature (37 C) seems most desirable since it is at that temperature that coagulation takes place physiologically An ordinary vacuum bottle filled with water at 3[illegible] C [illegible] a handy water bath for carrying out the coagulation time test The practice of perf[illegible] e tes[illegible] temperature should be abandoned since the temperature range in many laboratories [illegible] to 30 C and this causes considerable difficulty and confusion in interpreting the [illegible] deciding whether it is within normal limits

Th[illegible] of test tube The original Lee White procedure specified the use of a Widal [illegible] meter but test tubes of varying sizes are now employed To show the effect of size of [illegible] times of bloods in test tubes with internal diameters of 8 and 11 mm were compared [illegible] blood clot more rapidly in the smaller tube while hemophilic blood clotted con

THE VALUE AND THE LIMITATIONS OF THE COAGULATION TIME IN THE STUDY OF THE HEMORRHAGIC DISEASES

By Armand J Quick Ph D M D Rene Honorato C M D and Mario Stefanini M D

THE DETERMINATION of the coagulation time of the blood is among the most empirical procedures routinely employed in the clinical laboratory and is one most prone to be misinterpreted In a critical evaluation of this test one must consider first the mechanics of the procedure and second the physiological aspects which require a translation of an *in vitro* observation into a probable *in vivo* behavior that is coordinated with other factors bringing about hemostasis For this task a short historical survey is helpful since one can thereby acquire a knowledge of the evolution and development of the tests of coagulation time that are now in common use

Although delayed coagulation of shed blood in various conditions was observed even in antiquity there appears to have been no formal clinical test until 1878 when Vierordt[1] devised a procedure consisting of drawing a horse hair through blood in a capillary tube and observing first the point when fibrin threads adhered and again when the hair was free In 1893 Wright determined the coagulation time by filling capillary tubes with blood and noting the time when the contents could no longer be discharged by blowing This investigator appears to have been the first to state specifically that the coagulation time of hemophilic blood was delayed Brodie and Russell[3] three years later described a special instrument called a coagulometer in which a hanging drop of blood observed under a microscope is played upon by a current of air and the time determined for arresting the movement of erythrocytes In 1898 Hayem[4] introduced the simple procedure of putting venous blood in a test tube and noting how much time was required before a sufficient clot was formed to permit tilting without a flow of blood Fifteen years later Lee and White[5] employing the same principle devised a test which with minor modification has become the most widely used and most acceptable method for estimating the coagulation time It is this test which is critically studied in this paper

Several other tests should however be mentioned because they have in the past been employed extensively Two of these methods were described in 1904 The first was Burker s[6] in which a fine glass rod is passed repeatedly through a drop of blood thereby catching the first strands of fibrin formed The other was devised by Sabrazes[7] who filled capillary tubes with blood and at regular intervals of time broke off a short piece until a fibrin thread appeared between the severed sections Fuld and Schlesinger [8] in 1912 introduced another approach blood was placed in a U tube and the movement of a metal bead observed as the tube was gently tilted and the moment timed when the density of the clot fixed the bead A modification of this method by Hedenius[9] is still widely used especially in the Scandinavian

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This work was in part supported by a grant from the United States Public Health Service

countries For a detailed and complete review of this subject Nygaard s monograph[10] should be consulted

Interpretation of the coagulation time All the common procedures for determining the coagulation time are based on timing the interval between the removal of the blood from the vessel and the formation of sufficient fibrin to meet an arbitrarily designed end point The objective of every test is to measure the intrinsic coagulative power of the blood Obviously therefore trustworthy results can be obtained only by performing the test under constant and rigidly controlled conditions and by excluding all outside agents that influence the coagulation reaction Of the latter tissue juice is by far the most important since it contains thromboplastin It is therefore of utmost importance to exclude all traces from the specimen of blood used for determining the clotting time Obviously blood obtained by skin puncture (capillary blood) is utterly unsuitable since not only does it contain an appreciable amount of tissue fluid but even more serious the amount cannot be controlled Thus Christie[11] demonstrated that there were variations in the clotting time of the different drops of blood collected from the same puncture and Lee and White[5] cite the example of a hemophilic blood which had a coagulation time of 50 minutes for venous blood and only 5 minutes for capillary blood It must be emphasized that the coagulation time of capillary blood irrespective of the method used is worthless and unreliable for clinical purposes Even in taking blood by vein puncture enough tissue thromboplastin may occasionally gain entrance to reduce significantly the coagulation time as Jaques and his co-workers[12] have recently demonstrated It is not unusual to obtain a coagulation time of 8 to 10 minutes in a hemophilic subject whose true coagulation time is 1 hour merely by causing slight trauma in drawing the blood

Standardization of the coagulation time There is a distinct need for a simple and uniform procedure In the United States the Lee White test is gradually replacing the other methods This procedure is simple and yields as much and as accurate information as any test of coagulation so far devised but unfortunately the test has not been rigidly standardized and at present there is no strict uniformity in the details of the procedure

In order to devise a standard procedure it is necessary to consider the more important factors that influence the coagulation of blood in a test tube They are (1) temperature (2) size of tube and (3) the nature of the surface of the tube

Temperature Since the coagulation of blood involves chemical and enzymatic reactions it is obvious that the temperature must significantly influence the speed of the process This is clearly demonstrated by the results in table 1 in which the clotting times obtained at 22 C and 37 C are recorded The marked effect on hemophilic blood is particularly noteworthy Similar observations have been recorded by Patek and Stetson[3] The need for adopting a constant temperature at which the determination is carried out is evident Body temperature (37 C) seems most desirable since it is at that temperature that coagulation takes place physiologically An ordinary vacuum bottle filled with water at 37 C serves as a handy water bath for carrying out the coagulation time test The practice of performing the test at room temperature should be abandoned since the temperature range in many laboratories may be from 20 C to 30 C and this causes considerable difficulty and confusion in interpreting the clotting time and in deciding whether it is within normal limits

The size of test tube The original Lee White procedure specified the use of a Widal tube 8 mm in diameter but test tubes of varying sizes are now employed To show the effect of size of tube the clotting times of bloods in test tubes with internal diameters of 8 and 11 mm were compared Curiously normal blood clots more rapidly in the smaller tube while hemophilic blood clotted considerably faster in the

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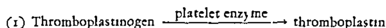
From the Department of Biochemistry Marquette University School of Medicine Milwaukee
This work was in part supported by a grant from the United States Public Health Service

It should be emphasized that the end point selected is arbitrary and does not mark the time of complete coagulation Unconverted fibrinogen may still be demonstrated A convenient way to measure incipient coagulation is to insert a glass rod coated with collodion into 1 cc of blood and then withdraw it gently every 30 seconds A fine thread of fibrin marks the beginning of coagulation In normal blood coagulation usually begins in 3½ to 4 minutes and is complete in 10 minutes whereas in hemophilic blood coagulation may begin (to cite a specific observation) in 10 minutes but require 2 hours more before enough fibrin is formed for a solid clot The tube with the glass rod should not be used for determining the final coagulation time

A New Theory of Blood Coagulation and its Bearing on the Coagulation Time

As the result of recent studies [14] evidence has been obtained to show that platelets do not furnish thromboplastin but in their disintegration liberate an agent probably an enzyme that activates thromboplastin which occurs in the plasma as a precursor and for which the term *thromboplastinogen* has been proposed It is probably identical with the antihemophilic globulin of Minot and Taylor and the prothrombokinin of Lenggenhager

According to this new concept the first step in coagulation can be expressed as follows

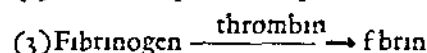


(1) Thromboplastinogen $\xrightarrow{\text{platelet enzyme}}$ thromboplastin

The activated thromboplastin reacts immediately



(2) Thromboplastin + prothrombin + calcium = thrombin



(3) Fibrinogen $\xrightarrow{\text{thrombin}}$ fibrin

The first and third equations are enzymatic whereas the second is stoichiometric Thus even a small number of platelets are sufficient to activate enough thromboplastin to furnish a quantity of thrombin that will coagulate blood within the normal period of time Such a quantity of thrombin may however be entirely inadequate as will be discussed later to meet the hemostatic requirements To understand the significance of the coagulation time it should be remembered that normal human blood could clot in 12 seconds if it had an optimum amount of thromboplastin and that furthermore the curve correlating the coagulation time and the concentration of thromboplastin is a hyperbola with both asymptotes zero This explains why the shortening of a coagulation time from 1 hour to 5 minutes can be brought about by an extremely small quantity of thromboplastin The amount of thrombin formed depends not on the coagulation time but on the quantity of prothrombin thromboplastin and calcium in the plasma and this can be looked upon as the key to a better understanding of several important hemorrhagic diseases which will be considered in this presentation

The coagulation time may be prolonged in four well known diseases or conditions hemophilia hypoprothrombinemia afibrinogenemia and heparinemia It is possible that a delayed coagulation may occur in other conditions but these have not been studied sufficiently to permit critical analysis Hypercoagulability remains a vague and as yet meaningless term

larger test tube (table 1) A possible explanation is that the clotting time is influenced by both the area of the surface in contact with the blood and the surface of the blood exposed to air In normal blood the disintegration of platelets is probably roughtly proportional to the amount of foreign surface and therefore the smaller the tube the *greater the surface and the faster* the disintegration of platelets In hemophilic blood the platelets in contact with a foreign surface do not readily undergo lysis and therefore the contact of the blood with air has a more predominating effect The influence of exposure to air is particularly well demonstrated by normal plasma deplateletized by high centrifugation Coagulation begins at or near the air plasma interface and travels downward

Since the size of tube definitely influences the speed of coagulation it becomes necessary to standardize by selecting a fixed size of tube Since nearly every laboratory is well supplied with serological test tubes (13 x 100 mm) and since this is a convenient size both for handling and cleaning it seems logical to select this tube for the test

TABLE 1 —*The Effect of Temperature Size of Test Tube and Surface of Test Tube on the Coagulation Time*

Subject		Coagulation time in minutes					
	Type of test tube	Glass	Glass	Glass	Lusteroid	Collodion coated	Silicone coated
	Internal diameter	11 mm	11 mm	8 mm	13 mm	11 mm	11 mm
	Temperature	22 C	37 C	37 C	37 C	37 C	37 C
Normal	I	13	6¾	4½	10½	26	47
	II	14½	6¾	5	14	23	41
	III	12	8	4½	12	29	52
	IV	10½	6¾	4¼	10½	27	43
	V	16	5½	4	16½	29	36
	VI	16	7	5	20	28½	42
Hemophilic	I	121	65	90	360	140	450
	II	76	25	31	190	240	340

Pyrex

Nature of the surface of the tube Blood clots fastest in glass as can be seen from the results in table 1 In a lusteroid (a synthetic plastic) tube coagulation is definitely delayed and in collodion coated tubes the retardation is even more marked Silicone (Dry Film) coating however is the most effective artificial surface known for preserving the fluidity of blood

A glass test tube is best suited for determining the coagulation time since as much information is obtained as would be by employing any other type of tube and it obviates the long waiting period which is always undesirable in a clinical laboratory

Recommended procedure for determining the coagulation time Blood is drawn by veni puncture preferably with a No 22 needle into a dry syringe If the determination cannot be made immediately a syringe coated with silicone (Dry Film) should be used and the blood kept in the syringe until the operator is ready for the test In drawing the blood the tourniquet should be applied just prior to the puncture If blood is not obtained immediately and without trauma another vein should be selected and a new puncture made One cubic centimeter of blood is transferred into each of 2 scrupulously cleaned test tubes (Pyrex 100 x 13 mm) Since the test tubes are apt to vary slightly in size only tubes having an internal diameter of 11 mm should be selected The tubes are immediately placed in a water bath kept at 37 C A vacuum bottle fitted with a cork having a hole in which the test tube can be inserted serves as a handy portable water bath The tube is gently tilted every 30 seconds and the end point taken as the moment when on tilting no flow of blood is any longer observed The normal range is 5 *to 10 minutes with the* majority of bloods clotting between 6 and 8 minutes

* Manufactured by the General Electric Company and marketed as Dry Film No 9987

by the coagulation time but only by the decrease in the prothrombin time so the assay of any antihemophilic agent cannot be made with an absolute degree of certainty by the coagulation time but will probably require the measurement of the prothrombin consumption

The coagulation time is obviously of limited value in hemophilia either in the diagnosis or in the treatment A prolonged value is suggestive of hemophilia provided other causes are ruled out A normal coagulation time does not exclude a diagnosis of hemophilia A history of bleeding and a markedly poor consumption of prothrombin during coagulation appears to be much more reliable evidence on which to base a diagnosis

The coagulation time is however of some practical and theoretical value A hemophilic with a coagulation time that is nearly normal usually has mild attacks of bleeding and only encounters serious trouble when relatively large vessels are damaged The severity of the bleeding tendency appears to be relatively independent of the coagulation time when the value of the latter exceeds 15 to 20 minutes In three hemophilics having average coagulation times of 25 55 and 120 minutes respectively the frequency and severity of the bleeding episodes during a period of observation of 6 months was roughly the same Theoretically the coagulation time is of value since it offers the only means to grade the severity of the hemophilic defect Thus the difference in availability of thromboplastin between the three hemophilics mentioned is so small that no other test including the prothrombin consumption can detect the difference

The coagulation time has it should be mentioned served not only in establishing the presence in plasma of an antihemophilic agent but has enabled Minot Taylor and their associates[15] to concentrate it They wisely depended not so much on a transient lowering of the coagulation time but on a sustained normal value

Hypoprothrombinemia Prior to the advent of vitamin K it was very puzzling to the surgeon why the jaundiced patient bled postoperatively in spite of a normal coagulation time The senior author on the basis of his early studies on vitamin K concluded that the hemorrhagic danger level was indicated by a prothrombin time of about 25 seconds which corresponds in man to a prothrombin activity of 20 per cent of normal At this level the coagulation time is so little increased that unless the test is done with great care it escapes detection since it is still well within the normal range In fact it has been found[16] that in dogs an increase of the prothrombin time from the normal of 6 seconds to 60 seconds is accompanied by a change of the coagulation time only from $3\frac{1}{2}$ to $5\frac{1}{2}$ minutes i e an average increase of only 2 minutes Even with extremely low concentrations of prothrombin the coagulation time is rarely as prolonged as in moderately severe hemophilia At very low levels the prothrombin time and the coagulation time tend to become identical Thus on reducing the prothrombin in a dog with dicumarol until the prothrombin time was 20 minutes a coagulation time of 19 minutes and a clotting time for recalcified plasma of 30 minutes was obtained The likely reason for such a result is that the limiting factor is prothrombin and that under such circumstances the thromboplastin of the plasma is adequate and therefore additional amounts of the latter have no further effect

The Coagulation Time in Hemorrhagic Diseases

Hemophilia With the exception of complete incoagulability of the blood as encountered in afibrinogenemia the most prolonged coagulation times are observed in hemophilia A coagulation time of one hour is not unusual but a time of two hours or more is rather infrequent provided the test is done carefully and at 37° C It has been found in recent studies that the coagulation time of a hemophilic may be surprisingly constant for a relatively long period of time Thus the coagulation time of one subject has remained about 55 minutes with few exceptions during the past 18 months Although it has been brought to normal several times with plasma transfusion it has always promptly returned to this rather fixed value The same constancy has also been found in other hemophilics but in no instance has the period been long enough to be significant

To understand the coagulation time in hemophilia it is necessary to understand the basic defect in this disease In a recent study[14] it has been found that hemophilic blood is almost completely devoid of thromboplastinogen and even after all the fibrinogen has coagulated no demonstrable consumption of prothrombin has occurred * All the coagulation is due therefore to a minute quantity of thrombin which is formed and which because it is an enzyme can convert all of the fibrinogen to fibrin in a relatively short time The minuteness of the quantity of thromboplastin which can bring about a normal coagulation time is clearly demonstrated by the following experiment

A stock extract of thromboplastin prepared by mixing 0 2 gm of dehydrated rabbit brain in 5 cc saline was diluted 1 to 1000 On adding 0 1 cc of this diluted thromboplastin to 1 cc of a hemophilic blood which had a coagulation time of 2 hours and 15 minutes the time was reduced to 5 minutes This 0 1 cc of thromboplastin contained only 2 5 gammas of solid material of which a large fraction was inert Obviously the amount of thrombin formed must have been extremely small yet it coagulated the blood in 5 minutes The conversion of prothrombin however was so small that it could not be demonstrated

From the results observed in hemophilia and in hypoprothrombinemia it seems definite that hemostasis is not dependent on the clotting time but on the quantity of thrombin supplied during the clotting process In hemophilia little thrombin is formed since the plasma lacks the thromboplastin precursor Even if the plasma contains enough thromboplastinogen to cause a normal coagulation time it may not be sufficient to supply enough thrombin for the hemostatic needs This explains why a normal coagulation time may be found in known hemophilics suffering from repeated hemorrhages In a limited number of such patients the senior author could demonstrate no consumption of prothrombin after coagulation had been completed Such patients are a problem to the surgeons since the normal coagulation may create a false sense of security Furthermore not every measure which reduces the coagulation time of a hemophilic is necessarily effective in controlling hemorrhage Just as the effectiveness of vitamin K cannot be established

* A new procedure named the prothrombin consumption test has been developed It consists in determining (by the senior author s method) the prothrombin remaining in the serum 1 3 and 24 hours after the blood has clotted

invariably occurred While there was a slight increase in coagulation time no clear cut relation between it and fatal hemorrhage could be found Interestingly, one of us has observed a series of cases with congenital hypoprothrombinemia and has found that those with a prothrombin time of 16 seconds showed no hemorrhagic tendency two cases with a prothrombin time of 19 seconds showed a distinct bleeding tendency and one case with a value of 30 seconds showed a very severe hemorrhagic condition

Obviously the coagulation time is of little or no value in the study of hypoprothrombinemia It cannot be used for controlling dicumarol therapy Again basically the fact is brought out that hemostasis depends on the amount of thrombin formed and when the prothrombin is reduced to about 20 per cent insufficient thrombin is furnished for stanching

Afibrinogenemia When total incoagulability of the blood is found afibrinogenemia should be suspected and a qualitative test for fibrinogen made Recently Pinniger and Prunty[18] demonstrated experimentally that the prothrombin time remained approximately normal in the blood of their patient until the fibrinogen fell below 50 mg per 100 cc of plasma and that the Lee White coagulation was 5 minutes when the fibrinogen concentration was as low as 30 mg It is obvious that the coagulation time has little practical value in this hemorrhagic condition except in the initial detection of a coagulation defect

Heparinemia Animals particularly dogs subjected to peptone or anaphylactic shock respond by an outpouring of histamine and heparin into the blood and by a marked thrombocytopenia The resulting heparinemia may be so great that the blood is rendered incoagulable In man the appearance of heparin in the blood has not been unequivocally demonstrated although there is a good probability that it can occur The increase of the coagulation time is not necessarily proportional to the concentration of heparin The latter can be much more accurately determined by titration with progressive dilutions of a standard thrombin solution [19]

The therapeutic use of heparin in the prophylaxis of thrombosis is successfully controlled by the coagulation time but this is entirely on an empirical basis since it has not been accurately determined how much heparin is needed for this purpose It is probable that the effective action of heparin consists in neutralizing thrombin and thus reduces the effective quantity of the latter

From the foregoing discussion it becomes clear that the coagulation time has limited value in the study of the known hemorrhagic diseases It has however an important function in the possible discovery of new hemorrhagic diseases On finding a prolonged coagulation a concise diagnosis can be made only by specific tests such as the prothrombin time and the prothrombin consumption test A little over a decade or two ago hemophilia was the waste basket for nearly all hemorrhagic diseases characterized by a coagulation defect Since then hypoprothrombinemia afibrinogenemia and heparinemia have been recognized as separate entities It is highly probable that other hemorrhagic conditions having a prolonged coagulation time exist but thus far have not been recognized and defined because of a lack of suitable methods of study

Early in the work on toxic sweet clover poisoning one of us[17] discovered that a heart puncture in a rabbit with a reduced prothrombin caused fatal hematopericardium This serves therefore as a useful means to study hemostatic effectiveness and

TABLE 2 —*The Relationship of the Coagulation Time Clotting Time of Recalcified Plasma and Prothrombin Time to the Hemostatic Breakdown*

Day	Rabbit	Prothrombin time	Coagulation time (Lee White†)	Clotting time of recalcified plasma	Effect of heart puncture‡
		sec	*min*	*sec*	
	1*	6	13½	185	
	2	6	13½	165	
	3	6	14	175	
	4	6	12½	165	
	5	6	14	180	
	6	6	12½	165	
1	1	6	13	170	
	2	11	14	240	
	3	12	15½	255	
	4	9	12½	210	
	5	11	15	225	
	6	10½	14	225	
2	1	6	14	180	
	2	25	15	450	Fatal hematopericardium
	3	19½	16½	405	
	4	13½	14	255	
	5	14½	15½	255	
	6	27	19	495	Fatal hematopericardium
3	1	6	13	175	
	3	24½	18½	450	Fatal hematopericardium
	4	18	15½	300	
	5	25	17½	420	Fatal hematopericardium
4	1	6½	13	180	
	4	24½	16½	435	Fatal hematopericardium

* Control the other five rabbits were given 2 mg per kg of body weight of dicumarol daily by stomach tube

† Blood was obtained from the median artery of the ear with a silicone coated syringe which probably accounts for the long normal coagulation time

‡ The heart punctures were made with a No 22 needle and always approximately in the same position

in table 2 a correlation is made between the prothrombin time coagulation time and the hemostatic breakdown A study of these results clearly shows that when the prothrombin time is less than 19 seconds the animals blood could prevent cardiac bleeding With a prothrombin time of 24 seconds or more hematopericardium

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SUMMARY

The coagulation time is a measurement of the intrinsic power of the blood to convert fibrinogen to fibrin It is an empirical test no matter how performed and therefore in order to be reliable requires that the test be done on venous blood under strictly controlled conditions A recommended procedure is outlined in detail

The coagulation time is prolonged in hemophilia hypoprothrombinemia afibrinogenemia and heparinemia In hemophilia the coagulation time theoretically is a measure of the severity of the disease but practically is of limited value since the coagulation time may be within normal limits in some patients the prothrombin consumed in the coagulation of hemophilic blood is therefore a better guide for diagnosis The coagulation time in hypoprothrombinemia is relatively little prolonged until a drastic reduction occurs The test is therefore of no value for establishing a hemorrhagic condition in hypoprothrombinemia In afibrinogenemia the blood is incoagulable A small amount of fibrinogen restores the coagulation time to normal

The presence of heparin increases the coagulation time The test is therefore useful in controlling the therapeutic action of this drug

The senior author in making a survey of the literature on hemorrhagic diseases in preparation of his monograph was impressed by the significant and diverse contributions which Dr George R Minot made to this field of medicine We feel honored to contribute this study to the collection of papers offered as a fitting tribute to Dr Minot who has so successfully and productively combined science and clinical medicine

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Method The cuff of a sphygmomanometer is placed around the arm and inflated to a pressure halfway between systolic and diastolic and maintained for three minutes in some cases and for five minutes in others reading the results in the area of a circle of 2 5 cm (about the size of a twenty five cent piece) with its center 4 cm below the bend of the elbow

Results In the 107 cases in which pressure was maintained for five minutes we found that 89 per cent had from 0 to 10 p techiae 7 4 p r cent showed 11 to 20 and 3 7 per cent (four cases) over 20 p techiae With three minutes pressure 54 tested cases showed from 0 to 4 petechiae Figure 1 shows that the results obtained with three minutes pressure give a shorter zone of normality and a more regular curve than those obtained with a pressure maintained for five minutes Further more five minutes pressure often produced great pain

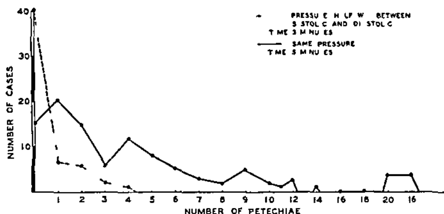


Fig 1 Tourniquet Test

According to our results we suggest for the tourniquet test the technic described above Normal subjects should show no more than 4 petechiae

Bleeding Time

The commonest testing methods for bleeding time are those of Duke and of Ivy There are slight modifications to the Duke technic [1] depending on the depth of the cut the size of the first drop and the care exercised in wiping away the blood [6 13] It is important to stress the fact that with Duke s method if the cut is deep enough to produce spontaneous bleeding the test is correct regardless of the size of the first drop of blood Chevallier s studies[14] confirm this statement Thus in this study we followed the original technic of Duke bearing the above facts in mind

Some authors have emphasized that in blotting up the blood the skin must not be touched with the absorbent paper Our results were obtained by completely wiping off the blood every 30 seconds for which we applied the absorbent paper directly to the puncture The contact with the skin was definite but gentle We think this is a better procedure otherwise blood may be left around the wound

HEMORRHAGIC TESTS STUDY OF 167 NORMAL SUBJECTS

By S ZUBIRAN M D AND L SANCHEZ MEDAL M D

DIAGNOSIS of the hemorrhagic disorders often depends upon the performance of a group of laboratory tests A patient with hemarthrosis excessive bleeding and history of hemorrhages in some members of his family cannot be diagnosed as being hemophilic until a definite abnormality in the clotting time of this blood is demonstrated in the presence of normal prothrombin determination

This situation demands special accuracy in the different tests used in the hemorrhagic disorders the necessity is thus evident for standardizing the procedures used for every test This is particularly important since great differences are found in the results obtained following the technics described by different authors

We were furthermore unable to find any report with results of these tests as performed in fairly large sample of normal controls In this study 168 normal adults of both sexes were selected Of these most were students with ages ranging from 16 to 48 years In practically all the cases the following set of tests was performed tourniquet test bleeding time blood clotting time clotting time of oxalated plasma on recalcification prothrombin time and clot retraction

In 27 of the studied cases there was a history of malaria but in none of them was there evidence of actual malaria or splenomegaly In 10 there was epistaxis in childhood probably of local origin The results of these cases were similar to the rest of the group and they will be considered altogether

Among the technics described by various authors we have selected those that in our opinion are relatively simple to apply and which offer at the same time possibilities of standard application These selected technics and the results we have obtained were as follows

Capillary Resistance

There are three ways of testing capillary resistance (1) tourniquet test (2) suction test (3) intradermal venom test With the last mentioned results are affected not only by the capillary resistance but mainly by the individual susceptibility to the venom This makes the test unsuitable for testing capillary fragility

The suction test is time-consuming and requires a special apparatus although very simple this is not usually available in most laboratories The test appears more precise than the tourniquet test[11] but the above disadvantages eliminate it for routine determinations

The tourniquet test is very simple and requires only a blood sphygmomanometer the results being accurate enough which accounts for our selection of it There are different technics for this test [1-5] the variables being the intensity of the pressure the time of applying it the area of reading the result and the number of petechiae considered as normal

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into a small glass test tube with an inside diameter of 8 mm rinsed with salt solution also The test tube is kept at room temperature and is given an endwise rotation every 30 seconds The end point is the time when the blood no longer flows from its position but maintains its surface contour at inversion of the tube

(2) Same as (1) temperature 37 C

(3) Same as (2) but dried material instead of saline rinsed material

In every case we started to count the time from the moment the blood appeared in the syringe

The curves of our results (fig 2) show the influence of the temperature and of the state of the tube walls At room temperature the curve obtained is flatter wider and displaced at the right in comparison with the curve of results obtained at 37

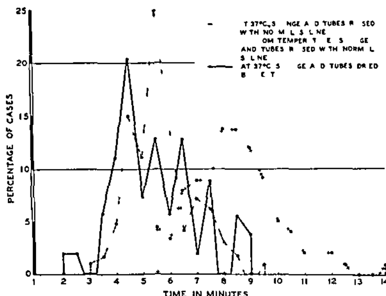


Fig 2 Results of the Clotting Time of the Blood in Normals

C The curve with dried material in comparison with that of rinsed tubes is more irregular and somewhat displaced to the o point of the time line All of them are very irregular Furthermore the blood coagulation time may vary widely as to normal limits (from 2 to 25 minutes) but its sensitivity as a test is rather low A normal clotting time of the blood as is well known does not mean normal coagulability of the blood We believe therefore that the blood clotting time is not a useful test in the study of the hemorrhagic disorders since it will not give undoubted data except in those cases with marked alteration in the clotting mechanism of the blood

Clotting Time of the Oxalated Plasma on Recalcification

For this test there are also several methods with which different normal results are obtained [4, 8, 16, 22] depending on the differences in temperature and on the

and may become clotted giving abnormally low bleeding times This difference in technic may explain the low normal figure (1 to 2 minutes) given by Schilling [13]

It seems unnecessary to standardize temperature for this test as suggested by Copley since it complicates the procedure and his normal figures [1] do not differ from ours obtained at room temperature with Duke s method

There are some interesting points in the use of Ivy s method In the first place punctures without bleeding are common around 10 per cent according to Ivy et al [10] who suggest that such punctures be disregarded and the test be repeated as many times as necessary to obtain three positive bleeding times taking the mean as the result Whenever the first puncture produced bleeding we reported it as the result but when it was zero we repeated the test usually obtaining a positive result and only occasionally requiring a third trial (3 per cent) of our cases

Occasionally a subject would ooze blood tinged serum after real bleeding stopped This oozing might obscure the end point It is advisable as Ivy says [10] to disregard such determinations making a new one

We think it convenient to use both methods simultaneously because some hemorrhagic patients may give normal results with one procedure and abnormal with another [6]

Results Duke s method In 136 cases (91 2 per cent) the bleeding time was three minutes or less it was three and a fourth to four minutes in 10 cases (6 7 per cent) and in only 3 cases was it four and a half minutes (2 per cent)

Ivy s method 130 cases (86 1 per cent) had three minute times or less 18 cases (11 9 per cent) had three and a half to six minute times and in only 3 (2 per cent) was it of six and a half or seven minutes

Our results suggest that the upper normal limit for the Duke s bleeding time be raised to four minutes since that figure will include a higher percentage of normal subjects (98 per cent) than the commonly accepted three minutes (91 2 per cent)

The commonly accepted upper normal limit for the Ivy bleeding time is six minutes although Ivy et al [10] give four minutes as the upper limit Since 15 of our cases (10 per cent) had bleeding times above four minutes and only 3 cases (2 per cent) above six minutes it seems more convenient to place the line of normality for this test at six minutes

Clotting Time of the Blood

There is a great number of methods to determine the clotting time of the blood discussions of them have been made by different authors [15, 16] The Lee and White method is the commonest of them but for itself there is a great number of modifications that make very difficult the interpretation of a given result They all have in common the use of venous blood and glass test tubes but a number of variations may be found from one to another of the Lee and White technics [17–20, 21]

Three methods were selected on the basis of their simplicity and were used to determine which of them was the most convenient

(1) Eight cc of blood are withdrawn from a vein using a sterilized syringe previously rinsed with 0 9 per cent salt solution One cc of this blood is drawn

same degree of activity and therefore it seems unnecessary to test each ampule of thromboplastin to obtain the normal standard

Clot Retraction

In studying this phenomenon we used the clots obtained in the determination of the blood clotting time In 108 cases two tubes were tested in one the clot adherent to the walls of the tube was destroyed mechanically by means of an

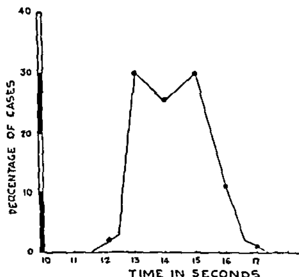


Fig 4 Prothrombin Determinations in 63 Normals

Table 1 —*Clot Retraction Results in Three Seri s of Normal Subj cts*

Tim afte blood clot was f rmed	At room t mpe atu						In w ter b th 37 C		
	Clot tub wall adhes n de tr yed			Cl t u tou hed			Clot t w destroyed		
	Ab nt	I compl t	Compl t	Ab ent	I complet	Compl t	Absent	I compl t	Compl te
how s	‰	‰	‰		‰		‰	‰	‰
One	9 2	90 8	0	42 5	57 5	0	0	78 8	21 1
Two	1 8	51 5	46 6	23 2	65	11 6	—	28 8	71 1
Four	0 9	32 4	66 6	6 5	53 7	39 8	—	5 5	94 3
Six	0 9	0 9	98	4 6	7 4	98	—	—	100

applicator and the other was kept without touching the clot Both tubes were left at room temperature In another series of 52 cases the adhesion of the clot to the tube was disrupted mechanically and the study was performed at 37 C placing the tubes in a water bath

The results are described in table 1 They show that normal clots kept at 37 C after mechanical disruption of their adhesion to the test tube develop retraction during the first hour in 100 per cent of the cases The retraction was complete in one hour in 20 per cent and in almost all of them (94 3 per cent) during the first four hours If the clots are placed at room temperature retraction starts during the first hour in only 90 per cent of the cases being complete in only two thirds of them

anticoagulants and recalcifying agents that are used We have used the following technic Four and five tenths cc of venous blood are mixed with 0 5 cc of 0 1 M sodium oxalate The blood is centrifuged at about 500 r p m for five minutes and finally the plasma is pipetted off One tenth cc of this plasma is mixed with 0 2 cc of 0 0 1 M calcium chloride solution in a test tube 8 mm inside diameter and the tube is placed in a water bath kept at 37 5 C The exact time required for the formation of a solid clot is recorded [4]

Results (Fig 3) One case had a time of less than 80 seconds (0 6 p r cent) 150 cases (97 5 per cent) from 80 seconds to 160 seconds and 3 cases more than 160 seconds (1 9 per cent) In every case two determinations were made and the average of them was the figure recorded

From these results we have the impression that the plasma clotting time is a more useful test in the study of the coagulability of the blood than the blood

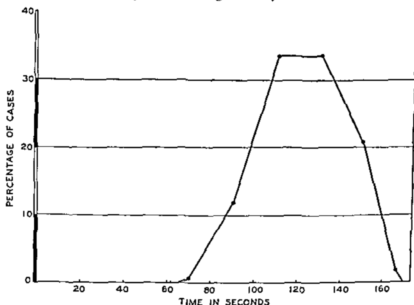


FIG 3 RESULTS OF THE PLASMA CLOTTING TI IE IN 154 NO IALS

clotting time This fact is shown by figures 2 and 3 The curve of the plasma clotting times in norm il subjects is far more regular than the curves traced with the results with whole blood It is also our impression that the plasma clotting time is a more sensitive test than the blood clotting time

Prothrombin Time

With Quick s technic double tests were performed in 63 normals using commercial Difco thromboplastin

The results obtained are illustrated in figure 4 This curve is quite similar to the Aggeler s graph illustrating the results in 30 normal persons [9] The actual normal range found was 3 seconds as found by different authors [3, 9] We suggest with Aggler testing each batch of thromboplastin with at least 5 controls taking the average as normal instead of running one control each time a prothrombin determination is made Difco thromboplastin has always shown in our exp rience the

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during the first four hours This delayed retraction is more marked when the adhesion of the clot to the tube walls is not disrupted mechanically in the latter situation some clots (4 6 per cent) will not show any retraction even after six hours

Mechanical disruption of the adhesion of the clot to the test tube walls and a constant temperature (37 C) are two important laboratory factors in the study of the clot retraction

Our results suggest that the syneresis of the blood clot be tested at 37 C disrupting at the start of the test the adhesion of the clot to the tube walls Following these conditions when a clot does not begin its retraction during the first hour or when it is incomplete after four hours the result of the test should be considered as abnormal

Conclusions

1 The standardization of the common laboratory tests used in the study of the hemorrhagic disorders is particularly important since there are many different technics with different normal figures for each test used

2 It is essential to select among the different technics used the simplest and most accurate for each kind of determination and to find the normal figures in a fairly large sample of normal subjects

3 A study was made of 167 normal individuals the simplest and most accurate technic being used for each test

4 From our results we suggest using the tourniquet test the Duke and Ivy bleeding time the prothrombin time and the clot retraction with the technics selected in this paper

5 Our study shows that blood clotting time determinations in glass test tubes are not very useful and their use unsuitable for clinical diagnosis

6 On the other hand the clotting time of the plasma gives more uniform results and its use appears more adequate than the blood clotting time in the diagnosis of the hemorrhagic disorders

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by borate at low temperatures. These insignificant traces of thrombin which form in solution may cause a slight fibrinous flocculation in four to six days (at storage). Owing to the broad zone of optimal fibrinogen concentration for clotting (Table 2) this trace of fibrin may be filtered off and the solution used for a day or two longer. However, this is not recommended in tests of the highest accuracy and it is unnecessary since a new batch of B.F. solution can be relied on to give identical clotting time values with similar thrombin solutions. Rigid tests with added $CaCl_2$ and thromboplastin show a trace of prothrombin, but this requires many hours to activate and then produces a mere trace of clot. By no criteria can these facts be regarded as in any way interfering with the data to be presented. Armour's B.F. is significantly free from trypase, tryptogen and enzyme inhibitors (v. infra). It is stated to yield about 60 per cent of fibrinogen (clottable N).

(b) *Human plasma Fraction-I* (H.F.) or antihemophilic globulin (Lot [illegible] Harvard Laboratory, courtesy Drs. E. J. Cohn, J. T. Edsall, G. R. Minot and colleagues) has a similar (6 per cent fibrinogen content), a somewhat (but not unduly) greater contamination with fibrinous and thrombin impurities, and a trace of active protease (trypase) together with significant amounts of its precursor (tryptogen)

Table 1.—*Data on Prothrombin Preparations*

Potency and stages of purification according to Seegers, W. H., Loomis, E. C., and Vandenbelt, J. M.: *Arch. Biochem.* 6, 85, 1945

Description		Stage puri.	Thrombic potency (specific activity)		Trace impurities (see t[illegible])			
Exp. tag	Lot No.		u. mg. tyrosine [illegible]	u. [illegible]	Thrombin	C	P[illegible]	Trypase
A	F101	Product 4	—	—	—	+	—	tr
B	S460904		15,000	—	+	tr	tr	o
C	S460315		5[illegible]00	—	tr	?	tr	o
D	S460424		9400	—	tr	tr	—	o
E	S461104		11,600	[illegible]000	+	tr	—	o
F	S4609[illegible]3		13,000	1400	—	tr	—	tr
G	S461219	Product 5	—	13[illegible]	tr	+	—	—
H	S4[illegible]04[illegible]1		9900	—	tr	tr	tr	o

Step[illegible] carried through ammonium sulfate and isoelectric precipitations, frozen and dried. (See Arch. Biochem. 6, 90, 1945)

which is best activated with Garner and Tillett's (1934) streptococcal agent now renamed streptokinase. In the present studies H.F. is used only as a source of plasma protease (q.v.)

3. *Prothrombins.* The data reported in this paper are obtained by the use of several prothrombin preparations purified to various stages of the method of Seegers, Loomis and Vandenbelt[41]. PRO A was prepared by ourselves from citrated dog plasma, while the others, PRO B–PRO H, were supplied by Dr. Seegers. A summary of data on these materials is appended in table 1 and their coagulant properties are discussed fully in the text. Brief allusion is also made to older Howell type[15] crude prothrombin precipitated by acetone from heat defibrinated (56 C) dog plasma, and dried on filter papers.

4. *Activators of prothrombin.* (a) *Calcium salt* (Ca). M/10 CaCl is prepared from stock M 1 (11.1 per cent) $CaCl_2$ by dilution, preferably with borate buffer.

(b) *Cephalin* (ceph.), the purified phospholipid isolated from brain,[15] is briefly alluded to in the text.

(c) *Thromboplastins* or *thromboplastin* (tpln.): the preparations repeatedly used in these studies are filtered (glass-wool) borate buffer suspensions of several commercial thromboplastins designed for use in plasma prothrombin assay tests, e.g., tpln. A Squibb's (rabbit brain), tpln. B Difco's (rabbit brain), tpln. C Sharp and Dohme's (horse brain). On a few occasions we used frozen dog brain (tpln. D). The universal finding of a trace of proteolytic enzyme in all these crude thromboplastins is noteworthy.

(d) *Thromboplastic enzyme*: see Protease

THE ACTIVATION OF PROTHROMBIN WITH SPECIAL REFERENCE TO THROMBOPLASTIC ENZYME (TRYPTASE)

By JOHN H FERGUSON M D , BURTON L TRAVIS B A , and EARL B GERHEIM M S

THE BASIC mechanisms whereby plasma prothrombin is converted into active thrombin in order to coagulate fibrinogen solution to fibrin clot can be shown by an experimental analysis of the isolated phases of clotting in vitro [23] The theoretic goal would be to isolate *prothrombin* in a chemically pure state and subject it to test reactions in order to learn the various factors which participate in its activation to thrombin In actual practice however the criteria of purity are difficult to define particularly in view of the fact that mere traces of certain factors[14 30] suffice to cause a considerable degree of activation if operating over a long enough period Such trace impurities quite fail to show up in the usual criteria e g constant solubility [61] unicomponent electrophoretic pattern and isoelectric point [6] and other data valid in protein chemistry [7] It is most necessary, therefore to supplement the examination of purified clotting agents with a series of sensitive tests for the presence and modes of action of these impurity factors

Seegers and colleagues[61] have carried the difficult problem of prothrombin purification to a point which is very satisfactory from the viewpoints of biochemistry and physiologic potency Through the courtesy of Dr Seegers we have been supplied with a number of these prothrombin preparations the experimental analysis of which provides the bulk of data herewith reported It must be emphasized that the results of these tests in no wise detract from the signal advance which these prothrombins represent in terms of potency and of freedom from all but traces of contaminants

I REAGENTS

1 *Borate b ffer* (buff) 45 vol 2 5 per cent H_3BO 45 vol 0 5 p r cent NaCl 10 vol 4 per cent $Na_2B_4O_7$ 10 H_2O (a simplification of the formula given by Burdon[3]) is used as solvent and diluent (e g to constant volumes) throughout It maintains a constant pH of 7 7 and largely controls the ionic strength of all our solutions Its mild bacteriostatic properties are valuable in p rmitting the prolonged keeping and observation of the protein solutions e g thrombin mixtures for several eeks and fibrinogen for many days at room temp rature and fibrinolytic tests (and controls) for similar periods at 37 C with only a very occasional contamination by molds and bacteria (e g T table 8) even without meticulous sterilization of glassware etc and more than simple corking of tubes to exclude dust and prevent evaporation

2 *Fibrinogens* (a) *Bovine plasma F action I* (B F) Armour Lab courtesy of Dr J B Lesh has proved to be a highly satisfactory fibrinogen preparation for quantitative clotting time studies An 0 5 per cent solution in borate buffer requires filtration of only a trace of insoluble fibrin like material Solutions should be kept at cool room temp rature (20 C) not in the icebox since the protein solubility is reduced

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b man serum a'b min (H S A) and *crystalline bovine serum albumin* (B S A) Harvard laboratory courtesy Dr J T Ed all [*] are found to be contaminated with a trace of active tryptase

(b) *Preparations containing enzyme precursor (tryptogen)* Many plasma protein fractions contain in addition to traces of active tryptase varying amounts of tryptogen (enzyme precursor) which can be activated by shaking with chloroform [44] or better by using streptokinase[4] (v infra)

(1) *H man pla ma Fraction I* (H F) v supra and the more refined (85–90 per cent clottable) but less stable (2) *h man fibri gen* (H Fb [12]) products of the Harvard Laboratory (courtesy Dr E J Cohn and colleagues) are repre entative of this group and so are (3) various ($(NH_4)_2SO_4$ pptd fibrinogens which we prepare from *dog* plasma (D Fb) On the other hand Armour Laboratory bovine fibrinogen and the more sati factory fibrinogen precipitated from bovine Fraction I (B F) by $(NH_4)_2SO_4$ are as enzyme free as the cruder fraction [23]

TABLE 4—*Effect of Keeping Prothrombin Solution with Reference to Activation and Thrombin Yields with Various Activators*

A *Activation series* C t (in seconds) at 25C pH = 7 - (borate buff) for 0 5 cc B F (0 5%) + 0 25 cc T

T 5 cc vol containing 4 cc PRO B (0 2%) + 0 25 cc M/10 $CaCl_2$ + 0 25 cc thromboplastic agent

T	D te	A t at	I cub t Pe od room tempe t e										Cl t l) (37 C)
			1 m	10 m	30 m	1 h	2 h	6 hr	1 dy	2 dy	4 dy	7 dy	
			seco ds										
1	10-8-46	Ca only	112	87	66	48	38	16	9	7	3	3	0 (10 dav)
2		Ca + tpln *A*	68	14	6	5	3	3	3	3	3	3	6–7 day
3	11-9-46	Ca + tpln *D*	20	12	8	4	3	3	3	3	3	3	not tested
4	10-8-46	Ca + *tryp* (4 u)	16	7	5	5	4½	6	19	47	100	—	3 day

B *Thrombin dil tion er s*

10–8–46 Undil T_2 2½ hrs old = 1 1 0 5 cc B F (0 5%) + 0 25 cc T_2 dilutions

R l T co	1 1	1 2	1 4	1 8	1 16	1 32	1 64	1 128	1 256
Clotting time in seconds	3	6½	9	18	25	39	61	90	230

(c) *P otease activators* The *st eptokinase* (strep) long miscalled streptococcal fibrinolysin used in the present studies is a 1 per cent extract (in borate buffer) of a potent preparation made by the Tillett and Garner method [27]

(d) *Trypt se* inhibito s Through the courtesy of Dr M Kunitz (Rockefeller Institute Princeton) e received some *cryst lline pancr atic tryp i inhibitor* (T I [*]) and *crystall n s ybean trypsin nh b tor* (S B I) the highly significant tryptase and thrombopla tin inhibiting properties of which are dis cussed in the text

II QUANTITATIVE METHODS

Owing to lack of methods for direct chemical analysis in dealing with such complex proteins as fibrinogen and prothrombin (or thrombin) reliable assay methods necessarily involve the whole coagulation reaction *Clotting time* (c t) measured under carefully standardized conditions [15] is the best and only currently practical solution to the problem of quantitative estimation of thrombin (*activated* prothrombin)

5 *Inhibitors of Prothrombin Activation* (a) *Citrate* (citr) 1/10 vol 4 per cent trisod citrate (hydrated) is optimal for (1) plasma preservation and (2) inhibition of spontaneous activation of prothrombin solutions (see text)

(b) M/1 *oxalate* ($K_2C_2O_4$) or *citrate* ($Na_3C_6H_5O_7$) added to an equal vol of thrombic mixture are best for progressive inactivation of thrombin intermediary (see text)

(c) *Heparin* (hep) 1 10 dil (borate buffer) of Lederle s Sod heparinate (1 per cent) gives a solution containing 100 Toronto units per cc which does not alter the pH of the buffer

(d) *Tryptase inhibitors* see *Proteases*

6 *Thrombin* For following fibrinogenolysis (e g table 13A) or clot lysis when the protease is to be studied in the fibrin it is necessary to use an enzyme free thrombin A convenient preparation for this purpose is a 1 per cent solution of lyophilized rabbit hemostatic globulin (h g) Lederle Laboratory courtesy Dr I A Parfentjev [54] Some of the products especially those supplied in solution for topical hemostasis [55] are less suitable however Other commercial thrombins viz (T_U) Upjohn s courtesy Dr J T Correll and (T_{PD}) Parke Davis courtesy Dr E A Sharp [62] are alluded to in table 20

TABLE 2 —*Clotting times in Relation to Concentration of Fibrinogen*

C t (in seconds) at 25 C for 0 5 cc fibrinogen (B F strengths* cited) + 0 5 cc thrombin (h g 1%)—see *Reagents*

Con (%)	2 0	1 0	0 5	0 2	0 1	0 05	0 0	0 01
Clotting time (in seconds)	6	5	5	6	7	10	47	115

* Grams of original material (B F 60 per cent clottable fibrinogen) per 100 cc

TABLE 3 —*Clotting times in Relation to Concentration and Age of Thrombin Solution*

C t (in seconds) at 20 C for 0 5 cc B F (0 5%) + 0 25 cc thrombin (T)*

Rel T conc (%)	100	50	25	10	5	1	0 5
			(T m r ec 1)				
Aged ½ min	4	8	11	22	41	84	127
30 min	4	8	11	22	48	104	194
60 min	4	8	11½	24	54	130	205

* T (100%) = 3 day old mixture of 4 cc PRO G (0 4%) + 5 cc buff + 0 5 cc tpln *D* + 0 5 cc $CaCl_2$ (M 10) — see T_6 table 8

7 *Proteases* (a) *Active enzymes* (1) Pancreatic *trypsin* (a) *crystalline trypsin* from pancreas courtesy Dr M Kunitz[44] (Rockefeller Institute Princeton) is desirable for such special purposes as distinguishing effects from those of chymotrypsin However for most practical purposes e g standard for routine protease assay by the fibrinogenolytic method [1] (b) *commercial trypsin* (Fairchild Bros and Foster) will suffice This trypsin (tryp) in the form of a 2 per cent extract made with equal vols of glycerol and borate buffer (*cf* Burdon[3]) filtered and stored in the ice box is the stock solution from which standard dilutions are freshly made (with borate buffer) immediately before use One tryptase UNIT is the fibrinogenolytic potency (for 0 25 per cent B F) of 0 01 mg (per cc) of our standard tryp in Lysis tests are conducted in the warm room (3 C) or water bath (39 C)

(2) *Tryptase preparations* (a) The Harvard human *plasma fraction* (III 3) supplied through the courtesy of Dr J T Edsall[9] has considerable proteolytic properties together with much thrombic activity (see text) (b) Dog *plasma tryptase* (trypt *D*) is prepared by a method we have not yet perfected (particularly from the point of view of stability of the enzyme preparation) but the product may be characterized as able to give complete fibrinogenolysis in 3 to 5 minutes and clot lysis in 9 to 0 minutes at room temperature when using 0 5 per cent B F or fibrin clot therefrom as substrate (c) *Crystalline*

the relative amounts of thrombin corresponding to the various clotting times The reservations with which we regard any clotting test system in which the over all *dilution* of the thrombin is changed led us to make the experiments of table 3

Thrombin instability during dilution experiments After complete activation of purified thrombins (see tables) the reproducibility of the clotting time values in test after test often over a period of several weeks, is (to anyone who has experienced difficulties in keeping the older types of unstable thrombins) truly amazing Yet any considerable dilution of these excellent thrombins at once introduces a factor of instability This is frequently apparent in less than a hour at room temperature and more rapidly if the temperature is increased [15] Table 3 shows thrombin dilution data first obtained immediately ($\frac{1}{4}$ min) after making the dilutions and subsequently repeated on the same solutions after $\frac{1}{2}$ hr and 1 hr respectively at 20 C The stronger thrombins show no detectible change but the higher dilutions weaken progressively

This phenomenon is encountered repeatedly with every type of thrombin studied including the purest preparations (free from protease for instance) Moreover any destructive impurity would be in highest concentration and presumably most active in the stronger solutions It makes no difference whether buffer or distilled water is used as the diluent There is no turbidization to suggest denaturation It can only be surmised that much remains to be learned about the way in which coagulant potency is related to the colloidal structure of the thrombin protein Over all *dilution* is undoubtedly a factor of considerable implication in the clotting system [16] Not knowing the extent of these implications we believe it safest to insist upon always using a definite amount of prothrombin in a *constant volume* of thrombic and clotting mixtures since this approximates most closely the natural clotting conditions

Prothrombin activation data presented (1) in many tables (4–16) as actual clotting times (c t) (2) (table 17) as computed thrombin percentages (rel T conc) or (3) (figs 2 3) graphically (activation curves) in the course of these studies are obtained as follows

A given amount of prothrombin (*fixed* for each series of comparative experiments) is mixed with any activators (and inhibitors) it is desired to study and the thrombic mixture (T) made up to *constant volume* with buffer solution Actually the most potent activator usually Ca is added last and the *incubation period* (i t) recorded from this moment At various periods a measured sample (usually 0 25 cc) is removed from the thrombic mixture and added to a labeled 12 mm diameter Wassermann tube containing the test fibrinogen (usually 0 5 cc 0 5 per cent B F) gently mixed and agitated and the *clotting time* (c t) noted with a stop-watch from the moment of mixing to the first appearance of definite fibrin threads

The *end point* in our water-clear solutions is sharp enough for an accuracy of 5 to 10 per cent which is often but a fraction of a second We ignore the earlier point of incipient turbidity and do not trust the later point of solidity (invertibility of tube) since this (a) is read with less accuracy (b) involves some nonsignificant variables (e g diameter of tube) and (c) is often incomplete in the case of extremely weak thrombins

Since there is only a minor (×3) dilution factor to the k continuing thrombin formation between adding the fibrinogen and the onset of clotting theoretical objection may be raised on this point That this is of little practical significance however follows from the fact that the most significant clotting

Experimental conditions[83] Being colloidal reactions[18] the clotting processes ar influ nced by (1) temperature (2) pH (3) salt content (4) concentration (dilution) of sp cific factors (v infra) and (5) adsorption and related colloidal phenomena[27] In the last category are (a) effect of wettable surfaces e g the well known ability of blood to clot quicker in glass than in paraffined[16 2] plastic[64] or silicone treated[61] tubes and (b) the clot aiding (second phase) or fibrinoplastic effects of a wide variety of nonspe ifi colloids e g kaolin[46] gum acacia[63] salmine[18] etc[44] The first step therefore is to standardize these experimental conditions

Fibrinogen concentration and clotting time Table 2 shows the varying clotting times when a given thrombin solution (1 per cent h g) is added to an equal volume (0 5 cc) of a series of fibrinogen (B F) dilutions Confirmatory of many older data[70] there is found a broad optimum which for this and similar fibrinogens,

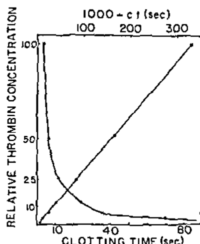


Fig 1 Clotting Times and Relative Thrombin Concentration (Percentage) The Inverse Law (ref Am J Med 3 67 1947)

Clotting times (sec) and 1000 ÷ c t (sec) of 1 cc fibrinogen (0 5% B F) + 0 25 cc thrombin (T) Temp 25 C pH = 7 7 (borate buffer)
T = 2 day old mixture of 8 cc PRO C (0 2%) + 1 cc buff + 0 5 cc tpln A (0 25%) + 0 5 cc M/10 CaCl

lies in the zone of concentrations between 1 0 and 0 25 per cent A stable thrombin repeatedly gives identical c t values with a recommended 0 5 *per cent* B F solution every time this is made from the lyophile dried plasma fraction

Thrombin concentration and clotting time Figure 1 shows the progressively shorter clotting times when a series of thrombin dilutions (0 25 cc vol) are tested on a given fibrinogen solution (1 cc 0 5 per cent B F) at pH = 7 7 (borate buffer) and room temperature (25 C) The reciprocals of the clotting times (1000 ÷ c t sec) in this particular experiment give a linear plot illustrative of the so called inverse law[1 35] In our experience this law is of limited application in a restricted range of thrombin concentrations and under experimental conditions which it is not possible to define It does not require rigid mathematical formulation however to grasp the fundamental fact that under standard test conditions a shorter clotting time means more thrombin Thrombin dilution series are given in a number of the following tables to indicate at least the order of magnitude of

months later on Dec 9 with another thromboplastin (tpln *D*) the prothrombin solution having been kept in the ice box and now showing considerable increase in thrombin content Nevertheless, it is apparent from the data that there is still much prothrombin requiring activation Although the activation is much slower in T_1 (Ca alone) it finally (4 days) reaches a stable optimum of thrombic potency which as measured by the 3" c t is identical with that in the other two series While therefore the prothrombin solution is unstable in the sense that it slowly changes to thrombin and the more rapidly the more favorable the conditions for activation yet it is amazingly stable in the sense of yielding a reproducible amount of thrombin (identical c t) even after two months storage and (ultimately) independent of the mode of activation used

The absence of *clot lysis* or even of *clot-r t action* in the T_1 series observed for 10 days at 37 C is noteworthy The extremely weak (6 to 7 day) fibrinolysis in the T_2 series confirms other data pointing to a trace of proteolytic enzyme in the thromboplastin preparation The possible significance of protease contaminants received special attention throughout these studies and the tests for lytic factors will be noted in the majority of the experiments There is no detectible trace of such factors in PRO B nor in the bovine fibrinogen (B F)

TABLE 6—*Stability of Prothrombin in Presence of Ca + and Thrombin*

T_{Ca} = 15 cc PRO F (0 35%) + 5 cc buffer + 1 cc $CaCl_2$ (M/10)

A = Activation data on thrombic mixture consisting of 2 cc T_C (1 min old at start) + 0 25 cc buffer

B = *Maximal* activation data selected from series of tests (but always reached within 1 hr) on thrombic mixtures consisting of 2 cc T_C (age cited) + 0 25 tpln (var fresh preparations)

C t (in seconds) at room temp (23 ± 2 C) 0 5 cc fibrinogen (0 5% B F) + 0 25 cc thrombic mixture

Age T_C	1 h	1 dy	2 dy	3 dy	8 dy	Cl t ly (37 C)
	Time e ds					
A	250	85	43	25	4	0 (7 day)
B	4	4	4	4	4	3-4 day

Stability of prothrombin during thrombin formation As we shall note more fully in a subsequent section Seegers purified prothrombins invariably show a trace of *active* thrombin immediately on making the solution A great deal more thrombin forms spontaneously on standing in solution at room temperature or in the ice box for several days The activating factors resposible for this will be brought out in the sequel In the data (table 4) just considered it is apparent that this thrombin can coexist in solution with unaltered prothrombin without demonstrable effect on the final thrombin yield The persistence of unaltered prothrombin still able to be activated and thus complete the (100 per cent) thrombin yield after 22 days at room temperature is recorded in the T_1* footnote to table 8 A In the following tables (5 and 6) are data to show that the same is true in the presence of amounts of (a) thromboplastin or (b) calcium salt that are ordinarily optimal for their respective roles in the activation process

times are measurable in a matter of seconds after a long (often hours or days) period of activation More over it tends to enhance the differences between a long clotting time (due to slow rate of thrombin formation) and a short c t (due to rapid approach toward complete activation) A larger dilution factor e g 0 1 cc T + 1.0 cc B F has been tried on a number of occasions without significant benefit Indeed it is less suitable for use with very weak thrombins and the volumes are less conveniently measured and pipetted

We have a number of objections to the use of oxalate or citrate in the fibrinogen[9] e g (1) continued thrombin formation is still possible especially in the presence of protease (see thromboplastic enzyme) (2) with unduly large amounts of anticoagulant (fig 2) there may be reversion of incompletely formed thrombin to prothrombin (see section on calcium and thrombin intermediary) (3) there is certainly some second phase inhibitory effect which may be excessive with very weak thrombins (compare T_1 and T table 8) (4) the c t end point is less sharp (a) in the case of oxalate because of opacity due to CaC_2O_4 (whenever calcium is present) (b) in the case of citrate because of undue translucency at the alkaline pH (7 7) of our borate buffer although 0 1 to 0 4 per cent citration is permissible at least with c t <30 sec without significant clot timing interference

It is to be emphasized that the significant differences between the activation (and other) data are readily appreciated by simple inspection

Second phase controls Whenever any question arises of possible clotting time modifications due to a reagent having some effect on the *second phase* (thrombin fibrinogen interaction) we always run suitable controls with several thrombins or thrombin dilutions Examples will be noted in the tables

TABLE 5 —*Stability of Prothrombin in Presence of (Brain) Thromboplastin*

X = 20 cc PRO D (0 2%) + 20 cc tpln *A* (0 25%) at room temperature

T= 4 cc X (aged as indicated) + 0 75 cc buffer + 0 25 cc Ca activated at room temp for periods cited (a t) Clotting times (c t) in seconds for 0 25 cc T + 0 5 cc B F (1%)

T	age X	1 m	10 m	20 m	30 m	1 h	3 h	4 hr	18 hr (a t)
		tim in seconds							
1	20 sec	334	29	9	6	4	3	3	3
2	24 hr	568	85	30	15	7	3	3	3
3	48 hr	640	126	39	19	8	5	4	3
4	72 hr	727	155	52	24	10	5½	4	3
5	Control	114	6	4	3½	3	3	3	3

Control 2 cc PRO D (72 hrs older) + 2 cc fresh tpln *A* (0 25%) + 0 75 buffer + 0 25 Ca

N B The initial prothrombin solution (in X) had been prepared 6 days previously and stored in ice box *Unfiltered* tpln *A* was used in T_4

III EXPERIMENTAL DATA

Stability of prothrombin in solution The tests of table 4 were made on a 0 2 per cent solution in borate buffer of PRO B which according to the stated (Seegers) potency of 15 200 thrombin units per mg tyrosine N (after activation) is one of the purest preparations Initial tests about 2 hours after making the solution show a trace of active thrombin estimated to be < 0 5 per cent of the total potential thrombin yield according to c t data in the accompanying *dilution series* (B)

Activation tests (A) were made initially on Oct 8 1946 with (T_1) Ca alone and (T) Ca + tpln *A* (0 1 per cent) The latter series was repeated (T_3) two

final potency of 3" (c t) in every case It can be concluded therefore that the prothrombin preserves its essential integrity in the presence of the crude brain thromboplastin

TABLE 8 —*Effects of Citrate and of Trypsin-inhibitors on Activation of Prothrombin*

A *Activation [illegible] inhibition data*

T 5 cc vol containing 2 c PRO C (0.4") with [illegible] Activators (0.25 cc M 10 CaCl [illegible] D) and Inhibitor (0.5 c [illegible] and final strengths noted [illegible] mg per c T)—see R [illegible]

Clotting times at room temp (23 ±2 C) 0.2"T × 0.5B F 0.5" after stated incubation period

T	Activators	Inhibitor	Incubation Period room temperature											Clot lysis (3° C)
			¼ hr	½ h	1 hr	2 h	4 h	1 dy	2 dy	4 dy	1 wk	2 wk	3 wk	
	(buff only)	0	4?m	44m	36m	33m	[illegible]m	[illegible]m	5m	4?m	130"	39"	15	0 (10 day)
2	"	citr (4.0	6m	5m	6m	3m	?m	6?m	69m	71m	61m	210"	5	0 (10 day)
3	Ca only	0	233	119"	1 5	150"	88	25"	29"	10"	6	4"	4	3 day
4		T I (0.[illegible])	220"	2 5	1??	1 4	115	?3"	22"	14	8½	5	4	4 day
5		S B I 0	336	33	340"	33	3??	223	??0"	315	??0"	5	1	0 (10 day)
6	Ca + tpln.	0	5	4	4	4	4	4	4	4	4	4	—	3 day
7		T I 0.5)	11	[illegible]	5½	4½	4	4	4	4	4	4	5	4 day
8		S B I 0.[illegible])	1 ?"	103	103	103"	115	110"	11?"	100"	43"	25	13"	0 (10 day)

Aft 1 hr incub with Ca + tpln., on 22nd day T was brought to 4 and T to 44 (but the T tube was contaminated by mold)

B *Thrombin dilution series*

0.5 B.F 0.5") + 0.25 cc T dilutions (T, 3 days old 10?")

R 1 T con [illegible]	100	50	25	10	5	1	0.5
Clotting time seconds	4	8	11	22	40	84	127

C *Second Phase controls*

Effects of citrate and trypsin inhibitors (same strengths as in A) [illegible] on thrombins (I–IV)

Thrombin		I	II	III	IV
		(seconds)	(seconds)	(seconds)	
Controls	"	3	7	?5	1 ?
Citrat	"	5½	7	1	119
T.I	"	5	6	19	—
S.B I	"	5½	7	21	—

The trace of protease in tpln *A* (shown by clot lysis tests of T in table 4) is evidently insufficient for any detectible prothrombinolysis [2]

B *Effects of calcium* The addition of optimal amount of calcium salt causes a significant but very minor increase in the slow rate of spontaneous thrombin formation and in the purest prothrombins studied full activation may not be obtained even in a week Table 6 shows such a recalcified prothrombin solution (PRO E) followed over an activation period of eight days At any intervening period as the data show it is merely necessary to add adequate amount of thrombo-

A *Effects of thromboplastin* The addition of thromboplastin alone is without significant influence on the spontaneous activation of purified prothrombin (see table 7 T_2) In the tests of table 5 0 2 per cent PRO D is mixed with an equal volume of dilute thromboplastin (tpln *A* 0 25 per cent) and kept at room temperature for three days Equal samples were recalcified (a) immediately (20 sec)

TABLE 7 —*Experimental study of certain activators of prothrombin*

A *Activation series*

T 5 cc vol containing 4 cc PRO F (0 2%) and when indicated ACTIVATORS e g 0 25 cc of M/10 Ca or tpln *A* (0 25%) and 0 5 cc of tryp (40-unit) or strep (1%)—see *Reagents*

C t (in seconds) at room temp (24 ± 2 C) 0 5 cc B F (0 5%) + 0 25 cc T (at incubation periods stated)

T	Activators	Incubation Period room temperature											Clot lysis (37 C)
		5 m	10 m	20 m	30 m	1 hr	2 hr	4 hr	1 dy	2 dy	3 dy	4 dy	
1	(buff only)	810	726	679	681	648	517	409	246	215	181	145	0 (10 day)
2	tpln only	880	863	803	807	830	811	809	411	288	226	186	0 (10 day)
3	Ca only	192	172	134	100	65	45	26	14	13	10	7	4 day
4	Ca + tpln	9½	5	4	3½	3	3	3	3	3	3	3	4 day
5	Tryp + Ca	10	6	4	4	4	6	13	27	58	140	—	3 day
6	Tryp + tpln	20	11	7½	5	5½	8	15	35	99	377	—	3 day
7	Tryp only	20	11	7½	5	5½	8	15	33	93	343	—	3 day
8	Strep + Ca	232	167	152	142	135	110	100	37	20	17	11	7 day
9	Strep + tpln	295	152	81	52	35	25	22	18	21	20	19	0 (10 day)
10	Strep only	397	325	352	360	343	330	360	197	155	90	65	0 (10 day)

B *Thrombin dilution series*

0 5 cc B F (0 5%) + 0 25 cc T dilutions (T_4 5 hr old = 1 1)

Rel T conc	1:1	1:2	1:4	1:8	1:16	1:3	1:64	1:128	1:256
Clotting time in seconds	3	4½	5½	13	24	43	80	180	287

C *Second phase control Effects of Streptokinase* Same conc as in A Various thrombins (I-IV)

Thrombin	0	I	II	III	IV
	cond				
Controls	∞	3	45	360	748
Strep	∞	3	26	230	320

and (b) after aging 24 48 and 72 hours respectively At the end of the series a control (c) was made up to contain the same amounts of prothrombin (now 72 hours older) calcium and a fresh thromboplastin suspension The prothrombin activation was followed in the usual way on each of the thrombic mixtures Except for the minor difference of a slower activation in T T_3 T_4 (probably explained by the thromboplastin in the mixture showing the same sort of deterioration as in ordinary solutions) the thrombin forming ability is unchanged and results in a

final potency of 3" (c t) in every case It can be concluded therefore that the prothrombin preserves its essential integrity in the presence of the crude brain thromboplastin

TABLE 8 —*Effects of Citrate and of Trypsin inhibitors on Activation of Prothrombin*

A Act at n (a d t h b t) d t

T 5 l t g 2 cc PRO C (0 4") with cited Acti t s (0 25 cc M/10 CaCl a d d l tpl *D*) and In h b t (0.5 c l an l fi l t e gths noted in mg per cc T)—see *R g f*

Clott ng t m at room temp (23 ± 2 C) 0 25T × 0 5B F (0 5") ft tated l ub t pe ods

T	Act ato	I h b t r	Incubat n Per od room t mperature											Clot lys s (3 C)
			¼ hr	½ hr	1 hr	2 hr	4 h	1 dy	2 dy	4 dy	1 wk	2 wk	3 wk	
	(b ff ly)	0	46m	44m	36m	33m	25m	7m	5m	4m	130"	39"	15	0 (10 day)
2		c tr (4 0)	76m	5m	76m	73m	72m	68m	69m	71m	61m	210	75	0 (10 day)
3	Ca ly	0	233	210"	1 8	150	88	23	20	10"	6	4	4	3 d y
4		T I (0 2)	270	215	198	1 4	115	23	22	14	8½	5	4	4 d y
5		S B I (0 2)	356	335	340"	335	36	223	250	345	290"	75	21	0 (10 d y)
6	Ca + tpl	0	5	4	4	4	4	4	4	4	4	4	—	3 d y
7		T I (0 5)	11	7	5½	4½	4	4	4	4	4	4	5	4 d y
8		S B I (0 2)	120"	103	103	103	118	110"	117	100	43	25	13	0 (10 d y)

Aft r 1 hr incub w th Ca + tpln on 22 d d y T w brought t 4 nd T t 44 (b t th T t be was c t m i at d by m 1½)

B Th mb d l t t

0 5 B F (0.5") + 0 25 T d l ti s (T₄, 3 days old 100")

R l T c (")	100	50	25	10	5	1	0 5
Cl tt g t me l seco ds	4	8	11	22	40	84	127

C S d Ph e c t ls

Effe ts f c t te and tryp h b t (am st e gth A) Va thromb ns (I IV)

Thromb		I	II	III	IV
		(ds)	(nd)	(eco ds)	
C trols	∞	5½	7	20	105
C t te	∞	5½	7	21	110
T I	∞	5	6	19	—
S B I	∞	5½	7	21	—

The trace of protease in tpln *A* (shown by clot lysis tests of T_2 in table 4) is evidently insufficient for any detectible prothrombinolysis [2]

B *Effects of calcium* The addition of optimal amount of calcium salt causes a significant but very minor increase in the slow rate of spontaneous thrombin formation and in the purest prothrombins studied full activation may not be ob tained even in a week Table 6 shows such a recalcified prothrombin solution (PRO E) followed over an activation period of eight days At any intervening period as the data show it is merely necessary to add adequate amount of thrombo

plastin (three different preparations were used on successive days) in order, within less than an hour, to bring the c t value to the same stable optimum (4 seconds) in every case This identical thrombin value is reached on the eighth day in the presence of added Ca alone Such reproducibility of thrombin c t value is taken to indicate (1) complete (100 per cent) activation of all the prothrombin and (2) perfect stability of the unaltered prothrombin and of the thrombin which it yields

C *Conclusion as to effects of thrombin* [19] From these three experiments therefore it is significantly concluded that the presence of thrombin during the intervening period i e prior to complete activation shows no demonstrable effects on the unaltered prothrombin either (a) destructive (*cf* [50]) or (b) as an autocatalytic [70] (?) activator (*cf* [1])

Mechanism of activation of purified prothrombins The data already considered suggest that the spontaneous activation of these purified prothrombins depends upon certain trace impurities By (a) controlling individual factors discovered to participate in the activation mechanism and (b) by testing for individual differences in behavior of the several prothrombin preparations much detailed information can be gathered as to the factors involved

Experimental study of certain factors in modifying the activation of a partially purified prothrombin Table 7 presents data on certain factors studied in relation to the activation of PRO F which is a fairly typical representative of Seegers prothrombin somewhat aged

(T_1) shows very slow and incomplete (4 day) spontaneous activation with buffer only (T_2) shows essentially no effect of thromboplastin alone (the slight retardation is not very significant) (T_3) shows a minor increase in activation on optimal addition of Ca salt alone although the 4 day c t value of 7″ denotes only about 20 per cent of complete activation (according to the thrombin dilution series B) (T_4) shows rapid (< 1 hour) and complete activation by Ca + tpln *A* (0 25 per cent)

Pancreatic trypsin (tryp) in a final concentration (chosen in an effort to minimize interfering proteolytic effects) of 4 units per cc of thrombic mixture is an excellent activator especially in the presence of Ca (T_5) In this experiment (*cf* fig 3) trypsin was also very effective when used alone (T_6) or with thromboplastin (T) i e without added Ca T_7 and T_6 are almost identical

The lengthening of the clotting times after the optimum in these three series is due to a *thrombinolytic* action of the trypsin Even the 1 –2 difference between the optima (as compared with the 3 c t optimum of T) may be significant of some *p othr mbinolysis* Speeding up of *fib inolysis* by a day in T_6 is significant and the 3-day lot ly is in T_6 and T_7 even more so (*cf* T_1 and T_2)

Streptokinase (strep) was included in these experiments (T_8 T_9 T_{10}) in order to control later tests (table 13) in which this agent was to be used as an activator of tryptogen (protease precursor) in certain plasma materials [33]

Tests show only minor effects which can be explained in two ways viz (1) some calcium content of the strep preparation (2) some speeding up of the second phase of the clotting process The latter is shown in the control tests C It is an example of the nonspecific effect of many adsorptive colloids the action of which we refer to as fibrinoplastic [8] In the absence of thrombin the streptokinase has no

coagulant properties (C O) Neither d xs it show proteolytic effects (T_4 T_{10}) but if anything (T_4) lessens the effectiveness of the fibrinolytic factor noted in T_3

Before going on to a detailed consideration of the individual prothrombin activators we shall present some data on the effects of certain *inhibitors* because of the light they shed upon the activator mechanisms

Effects of certain inhibitors on activation of purified prothrombin

(Tables 8 and 9) *Citrate* (citr) The spontaneous activation of these prothrombin solutions is very greatly inhibited by 4 mg per cc (final concentration) of trisodium citrate Only after a week is there evidence of a trace of activation but this is still barely noteworthy at the end of two weeks and only 1 per cent (approximate) in three weeks (table 8 T) The persistence of unaltered prothrombin on the twenty second day is shown by incubating the residual T_1 T_2* with Ca +

TABLE 9 — *Inhibitory Effects of Heparin D i g Prothrombin Activation*

T_1 = 4 cc PRO E (0 35%) + 0 5 cc buffer soln + 0 25 cc tpln *A* (0 25%) + 0 25 cc M/10 $CaCl_2$

T_2 = 4 cc PRO E (0 35%) + 0 5 cc hep (100 unit) + 0 25 tpln *A* (0 25%) + 0 25 cc M/10 $CaCl_2$

Clotting tests 1*a* — 0 25 T_1 + 0 25 buffer soln + 0 5 B F (1%)
1*b* — 0 25 T_1 + 0 25 hep (10 unit) + 0 5 B F (1%)
2 — 0 25 T_2 + 0 25 buffer + 0 5 B F (1%)

T_3 = 4 cc PRO + 0 25 buffer + 0 25 Ca + 0 5 *trypsin* (10 unit) incl for future reference (table 18)

Th mb M t e (T)	5 m	15 m	30 m	1 h	2 h	8 h	1 dy	2 dy	3 dy	7 dy
1*a* (*without* heparin)	63	42	20	9	6	4	4	4	4	4
1*b* (2nd phase control)	840	60	29	12	8	5	4½	4½	4½	4½
2 (*with* heparin)	2 hr	375	60	19	13½	10	9	8	7½	4½
3 (with trypsin)	32	—	8	5	4	4	8	14	40	—

tpln for 1 hour T_1 is brought to the complete c t of 4 seconds T_2 fell only to 44 seconds and proved unstable (48 seconds in 4 hours) but the obvious growth of a *mold* in the solution could explain the lysis of prothrombin (and thrombin) In a very similar but unspoiled test T_1 of table 16 the citrated PRO G was brought to the full c t 4 seconds value on the seventeenth day (v p 1149) The citrated prothrombin from the start shows a faint trace of coagulant activity which is the best evidence that the prothrombin actually contains this as a trace impurity and not as the result of activation proceeding only in solution

Crystalline pancreatic trypsin inhibitor [5] (T I) table 8 T_4 shows that 0 2 mg per cc (final concentration) of crystalline pancreatic trypsin inhibitor is almost without effect on the activation of prothrombin by Ca alone while 0 5 mg per cc has only a slight delaying action on the activation by Ca + tpln

(The enzyme inhibitor does delay clot lysis by about a day (37 C))

Crystalline soybean trypsin inhibitor[41] (S B I) in final concentration of 0 2 mg per cc is markedly inhibitory to the activation of prothrombin both by Ca alone (T_5) and by Ca + tpln (T_8) Its inhibition of the fibrinolytic enzyme is complete

The differences between S B I and T I may be significant suggesting that the soybean inhibitor has certain direct antithromboplastic (? anticephalin) effects not seen (to any important extent) with T I and probably unrelated to its effects on any protease present

The *second phase effects* of citrate and the trypsin inhibitors are negligible in the control tests (table 8 C)

Heparin[31] (Hep) It is important to note Seegers claim[61] that his purified prothrombins are antithrombin free and there is no reason therefore to suspect that heparin as used in the tests of table 9 is acting other than directly i e in the absence of co-factor or heparin complement In the amount used (100 units = 1 mg per cc) heparin has some antithrombic inhibitory (clot de

TABLE 10 —*Effects of Varying Amounts of Ca^{++} During Prothrombin Activation*

T thrombic mixtures 5 cc vol containing (in borate buffer) 1 cc PRO H (0 1%) + 0 25 cc tpln *B* (0 5%) + 0 25 cc $CaCl_2$ (final strengths cited) Clotting times (sec) at 29 C, pH = 7 7 for 0 25 cc T + 0 5 cc B F (0 5%) + 0 25 cc *diluent* (containing same amount of tpln *B* as in T and exactly enough $CaCl_2$ to bring to same final conc (0 0125 M) in *each* clotting (T + B F) mixture

T	Ca in T	½ m	5 m	10 m	15 m	30 m	1 hr	2 hr (t)
		seconds						
1	0	127	126	125	125	129	139	145
2	0 002M	104	12	4½	4	4	4	4
3	0 005M	99	6	4½	4	4	4	4
4	0 025M	98	6	5	4½	4	4	4
5	0 05 M	139	75	52	21	7½	5	4

laying) effect on the *second phase* which can be seen by comparing series 1*b* (in which heparin was added to the fibrinogen in amounts exactly corresponding to those entering the clotting mixtures in series 2 with the heparin free mixtures of series 1*a* It is noteworthy that this antithrombic action is most marked in the case of the weak initial thrombins but almost negligible (½ second) in the powerful fully formed thrombins at the end of the series Series 1*b* therefore is the proper control for the *first phase* action of heparin which series 2 shows to consist in a marked delaying (antiprothrombic in a general sense)[13] action on the prothrombin activation After a week however the 4½ seconds c t is identical with the control proving that there is no difference in the ultimate thrombin yield These results closely resemble those obtained[13] by a slight reduction of the thromboplastic factor (table 11) and are best termed antithromboplastic [13] (*cf* [5])

(From data published elsewhere [33] it is established that heparin acting alone is unable to inhibit the fibrinolytic enzyme)

Consideration of individual prothrombin activators

I CALCIUM

The ability (a) of citrate (or oxalate etc.) to prevent the spontaneous activation of prothrombin solutions and (b) of added *ionized* Ca salt to speed up thrombin formation to a degree depending upon certain thromboplastic factors (see below) confirms the long established fact[11] that ionized calcium (Ca^{++}) is ordinarily essential for the activation of prothrombin to thrombin.

Amount of calcium present. Paucity of materials precluded any attempt at quantitative Ca analysis of these prothrombins particularly since the inability to improve the extremely slow spontaneous activation by simply adding thromboplastin (e.g. table 7 T) was believed to indicate that very little calcium could be present. However a qualitative test in which 1 cc of 0.4 per cent PRO G was treated with an equal volume of M/1 $K_2C_2O_4$ showed a definite turbidity and overnight sedimentation of a trace of calcium oxalate. In this particular preparation

TABLE 11.—*Effects of Varying Thromboplastin Concentration on Activation of Prothrombin with Special Reference to Thrombin Yield*

5 cc vol. of (T) thrombic mixtures (A–E) containing 2 cc PRO A + 0.25 M/10 CaCl + tpln. *A* (final dilutions stated) in borate buffer (pH = 7.7). C.t. (sec.) at room temp. (25 ± 2 C). 0.5 BF (1%) + 0.25 T

Thromb. Mixture	A	B	C	D	E
Conc. of thromboplastin	1:500	1:1000	1:4000	1:40 000	1:400 000
Rel. conc.	800	400	100	10	1
Optimal thrombin c.t.	4	4	6	8	10
Time needed to reach optimal activation	1 hr	2 hrs	18 hrs	3 days	4 days
CLOT LYSIS (at 37 C)	3 day	5 day	>7 day	0 (7 day)	0 (7 day)

Good clot retraction but lysis incomplete

therefore contamination with some available calcium was actually demonstrated. PRO G was used in the experiments of table 7 but neither in this case nor in the data of similar studies (see tables) can the test with buffer alone be taken as evidence of differences in the amount of calcium operating (as compared with the recalcified mixtures) since the thromboplastic factor is also a variable. Only when thromboplastin alone is added as in T_2 of table 7 without *improving the activation can it be suggested that inadequate amount of available calcium (in PRO F in this case) is responsible for the extremely slow and inadequate thrombin* formation. It is not possible in the 4 day observation period of this particular experiment to predict the ultimate thrombin yields in T_1 and T_2. The mode of action of thromboplastic enzyme is discussed later (pp. 1146–1150) but it may be stated from the data of table 7 that *trypsin* appears able to make both calcium and thromboplastic factor (prob. cephalin) available from otherwise inert combinations with the prothrombin protein (T_6, T_6, T).

Effects of varying Ca^{++} concentration in first phase of clotting. (Table 10.) It has long been recognized[11] that the essential action of Ca ions in the clotting process is

Crystalline soybean trypsin inhibitor[41] (S B I) in final concentration of 0 2 mg per cc , is markedly inhibitory to the activation of prothrombin both by Ca alone (T_6) and by Ca + tpln (T_8) Its inhibition of the fibrinolytic enzyme is complete

The differences between S B I and T I may be significant suggesting that the soybean inhibitor has certain direct antithromboplastic (? anticephalin) effects not seen (to any important extent) with T I and probably unrelated to its effects on any protease present

The *second phase effects* of citrate and the trypsin inhibitors are negligible in the control tests (table 8, C)

Heparin[31] (Hep) It is important to note Seegers claim[61] that his purified prothrombins are antithrombin free and there is no reason therefore to suspect that heparin as used in the tests of table 9 is acting other than directly i e in the absence of co-factor or heparin complement In the amount used (100 units, = 1 mg per cc) heparin has some antithrombic inhibitory (clot-de

TABLE 10 —*Effects of Varying Amounts of Ca++ During Prothrombin Activation*

T thrombic mixtures 5 cc vol containing (in borate buffer) 1 cc PRO H (0 1%) + 0 25 cc tpln *B* (0 5%) + 0 25 cc $CaCl_2$ (final strengths cited) Clotting times (sec) at 29 C pH = 7 7 for 0 25 cc T + 0 5 cc B F (0 5%) + 0 25 cc *diluent* (containing same amount of tpln *B* as in T and exactly enough $CaCl_2$ to bring to same final conc (0 0125 M) in *each* clotting (T + B F) mixture

T	Ca T	½ m	5 m	10 m	15 m	30 m	1 hr	2 hr (t)
		seco ds						
1	0	127	126	125	125	129	139	145
2	0 002M	104	12	4½	4	4	4	4
3	0 005M	99	6	4½	4	4	4	4
4	0 025M	98	6	5	4z	4	4	4
5	0 05 M	139	75	52	21	7½	5	4

laying) effect on the *second phase* which can be seen by comparing series 1*b* (in which heparin was added to the fibrinogen in amounts exactly corresponding to those entering the clotting mixtures in series 2 with the heparin free mixtures of series 1*a* It is noteworthy that this antithrombic action is most marked in the case of the weak initial thrombins but almost negligible (½ second) in the powerful fully formed thrombins at the end of the series Series 1*b* therefore is the proper control for the *first phase* action of heparin which series 2 shows to consist in a marked delaying (antiprothrombic in a general sense)[19] action on the prothrombin activation After a week however the 4½ seconds c t is identical with the control proving that there is no difference in the ultimate thrombin yield These results closely resemble those obtained[13] by a slight reduction of the thromboplastic factor (table 11) and are best termed antithromboplastic [17] (*cf* [5])

(From data published elsewhere [33] it is established that heparin acting alone is unable to inhibit the fibrinolytic enzyme)

thrombin formation proceeds via an intermediary (calcium prothrombin cephalin) complex or compound [1] Although this idea seems to have been well received (ref [70]) the intervening decade has not brought forth confirmatory publications from other laboratories We have therefore taken the present opportunity to repeat the same kind of experiments with the newer highly purified prothrombins Fig 2 reproduced from a recent review [79] shows what we believe to be a typical result In this experiment a control (I) thrombic mixture (PRO C (0 5 per cent) + Ca + tpln) was followed without oxalate A portion of the same mixture was removed (a) after 5 minutes (3) and again (b) after 30 minutes (4) in each case being added to an equal volume of M/1 $K_2C_2O_4$ and the further activation studied by using *double the volume* of the new mixture in the clotting test A second phase control (2) consisted in adding to the fibrinogen a *single volume* of the original thrombic mixture shortly (60 sec) after the addition of a like volume of M/1 $K_2C_2O_4$ Buffer was used instead of oxalate in 1

Under these fully controlled conditions therefore it is highly significant that the earlier (5 min) oxalation (3) of the prothrombin thrombin mixture showed *progressive loss* of thrombic potency in marked contrast to the riper (30 min) mixture (4) which is unaffected by addition of oxalate Our earlier paper[12] may be consulted for evidence that the final thrombin may be decalcified by oxalation and electrodialysis without significant loss of thrombic potency

II THROMBOPLASTIC PHOSPHOLIPID

A *Cephalin* (ceph) The phosphatide or more accurately the group of phospholipids (since there are isomers and homologues with varying fatty acid radicals not all of which are clot aiding[14]) designated chemically as *phosphatidyl ethanol amine* [35] is a thromboplastic agent At least this is true of the P lipid as isolated from brain and other tissues as well as from plasma etc the fact being first clearly recognized by Howell[39] and Zak[71] in 1912 although the identification of the phosphatide required later work and may still be incomplete [1, 4] In some earlier quantitative studies[14] on crude (Howell type) prothrombin we showed (1) as little as one part of cephalin in several millions has distinct thromboplastic effects (2) thrombin yield depends upon amount of free (or available) cephalin present (3) considerable amounts of cephalin (and other substances) can be demonstrated in combination with the prothrombin protein[10] even when it shows little if any ability to be activated by Ca alone [30]

It would be highly desirable to repeat these data especially (a) the analytical and (b) the full study of thrombin yields upon the newer prothrombin preparations At present however we are handicapped by lack of cephalin and of sufficient prothrombin With two old (several years) and weak cephalin preparations we were able to confirm the thromboplastic action of the P lipid but this is the extent of the data which for the present we can include under this head

B *Thromboplastin* (tpln) We have had no opportunity to study the *thromboplastic lipoprotein* which Chargaff et al [4] claim to have isolated from lung tissue and plasma The present data were obtained with the use of various crude *thromboplastin* (*thrombokinase*) preparations described under *Reagents* Mertz Seegers and

limited to the first phase (activation of prothrombin) and requires a Ca^{++} concentration above a certain minimum and not in excess of a certain optimum because of inhibitory effects [14, 47] The exact working out of the effects of varying the amount of calcium during first phase tests is rendered difficult [9] because of a significant second phase action at least with higher Ca concentrations due to relatively nonspecific ion effects during the thrombin fibrinogen interaction [35] The experiments summarized in table 10 represent a new attempt to clarify this problem

All reagents including CaCl , were dissolved in borate buffer The (added) Ca content was varied from 0-0 05M (T_1–T_5) and the amount of thromboplastin was kept constant throughout A series of *diluents* was prepared containing the same amount of thromboplastin and exactly enough $CaCl_2$ for admixture with an equal volume (0 25 cc) of the respective thrombic mixtures and 0 5 cc B F to

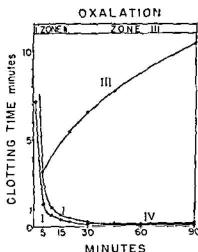


FIG 2 EFFECTS OF OXALATE AT VARIOUS STAGES (ZONES) OF PROTHROMBIN ACTIVATION (ref Am J Med 3 67 194) Clotting and incubation times at 25 C pH = 7 7 (borate buffer) I Thrombic mixture (T) 13 5 cc PRO C (0 5%) 0 75 cc tpln A (0 25%) + 0 75 cc M/10 CaCl Clotting test 1 cc B F (1%) + 0 25 cc buff 0 25 cc T_1 II Clotting tests 1 cc B F (1%) + 0 25 cc M/1 $K_2C_2O_4$ + 0 25 cc T_1 III Thrombic mixture (T_2) 3 cc T_1 (5 min old) + 3 cc M/1 $K_2C_2O_4$ Clotting tests 1 cc B F (1%) + 0 5 cc T IV Thrombic mixture (T) 3 cc T_1 (30 min old) + 3 cc M/1 $K_2C_2O_4$ Clotting tests 1 cc B F (1%) + 0 5 c T_3

bring the Ca concentration in the final clotting mixture to 0 0125M in every test The control of Ca and tpln in the second phase is therefore complete The optimal 4 seconds c t was identical in T_1–T_4 and no difference was noted in another test not included in the table with T_3 + buffer (instead of diluent) which reduced the Ca concentration to 0 00125M Thus in a range of Ca concentration without second phase effects there is seen (table 10) marked variations in the *rate* of prothrombin activation but no effect on the *amount* (potency) of thrombin ultimately formed For this first phase action of Ca salt there is a definite optimum at about 0 005M (T_3) with delayed activation below this (T) and an inhibitory slowing at higher Ca^{++} concentrations (T_5)

Effects of oxalate (or citrate) during prothrombin activation The thrombin intermediary In 1937 we published some experiments in support of the view that

plasmin[8] or fibrinolytic protease[47]) However this is not regarded as a basic factor since prothrombin in our experimental systems is rapidly and completely converted into thrombin by calcium (Ca^{++}) and cephalin (thromboplastic phospholipid) even when no protease can be demonstrated We had shown this previously[20] when using crude prothrombin Trypsin in vitro corrects the thromboplastic defect of hemophilia[18] In the present study all the thromboplastic preparations (except cephalin) showed at least a trace of tryptase impurity revealed by the clot lysis test The role of the thromboplastic enzyme we believe to be of the nature of a weak digestive action (disaggregation Pope[57]) upon the complex unions of plasma proteins with phospholipid and calcium thereby *mobilizing* or making available these necessary activators and thus in a sense catalyzing the prothrombin activation The high fidelity with which the new purified prothrombins permit us to follow the course of the activation process has enabled us to contribute the following highly significant data on (1) the thromboplastic activity of natural tryptase in a variety of plasma products and (2) certain interrelationships between thromboplastic and proteolytic properties Highlights of these data are (3) use of streptokinase (v infra) instead of the less reliable Delezenne and Pozerski[66] (1903) chloroform method for activation of protease precursor (tryptogen) and (4) study of effects of certain recently isolated crystalline trypsin inhibitors of protein or polypeptide nature from pancreas[5] (T I) and soybean[44] (S B I)—see *Reagents*

Thromboplastic action of tryptase (streptokinase activated tryptogen) in human and other fibrinogen containing plasma fractions Our first clear demonstration of the thromboplastic action of natural plasma tryptase was made with streptokinase activated human plasma Fraction I (H F) and the more refined fibrinogen (H Fb) obtained from the Harvard laboratories (see *Reagents*) The data of table 13 are reproduced with minor modifications (including clot lysis data) from Proc Soc Exper Biol & Med *64* 312 1947[21]

The preliminary *fibrinogenolytic tests* (A) show that streptokinase (strep) activates the plasma tryptogen in H F to a proteolytic potency comparable to that of 20-unit (final concentration = 2 units per cc) trypsin (tryp)—(see *Reagents*)

The thrombic mixtures for the activation tests (B) consist of 5 cc vol containing 2 cc PRO B (protease free 1 T_1) 2 5 cc of lysate (or buffer) + 0 25 cc buffer + 0 25 cc Ca Thus the trypsin concentration (final) in T_4 is only 1 unit (= 0 01 mg) per cc Obviously the amount of protease in these activation tests is very small indeed

The activation data in the presence of optimal calcium (added in all tests) clearly show the thromboplastic improvement in thrombin formation by the *lysates* A (tryptase) and B (trypsin) in T_2 T_4 respectively Heat defibrinated H F (C) is used to control the possibility of some thromboplastic factor other than the proteolytic agent being the cause of the T_2 result The series T is practically identical with series T_1 (Ca only) Thus we conclude the enzyme acts in conjunction with the trace of thromboplastin mobilized from the prothrombin preparation

We have previously noted (table 7) that the *streptokinase* (protease activator) is devoid of significant thromboplastic thrombic or proteolytic effects

Smith[50] found that the amount of added thromboplastin (a lung preparation) determined the thrombin yield from purified prothrombin Table 11 shows the essential data when we follow for 7 days the effects of varying concentration of brain thromboplastin (tpln *A*) added to recalcified dog prothrombin (PRO A) a partially purified preparation The least amount of the tpln in the thrombic mixture was 1 400 000 which was weak but by no means minimal The 1 1000 and 1 500 concentrations are clearly maximal Owing to the limited amount of prothrombin available there was insufficient thrombin for a dilution series but it is clear nevertheless that (1) above a certain maximum increasing the thromboplastin concentration speeds up the *rate* of prothrombin activation without increasing the thrombin *yield* (as shown by c t value) whereas (2) below a certain optimum lessening the thromboplastin definitely reduces the final amount of thrombin formed in addition to greatly slowing the rate of activation Thus the main facts, previously reported with regard to the thromboplastic action whether of cephalin[14] or crude thromboplastin [50] are adequately confirmed in the present study

The data of table 11 again clearly show the trace of fibrinolytic factor which since it diminishes with the thromboplastin concentration must come chiefly from this source

Deterioration of thromboplastic impurity in prothrombin preparations During the course of these investigations we had occasion to secure prothrombin activation data of similar kind on repeated experiments with the same prothrombin preparation over some weeks or months It became apparent that a change in the character of the activation could often be noted An especially good example of this is seen in data obtained as little as three weeks apart, in the case of PRO G as is clearly seen from the data collected in table 12 The rate and degree of spontaneous activation are particularly affected (*1a* vs *1b*) but there is also much weakening of the ability to be activated by Ca alone (*2a* *2b*) Even the better start which the Ca gets in a repetition (*2c*) of the last test after 24 hr keeping the PRO G solution (in ice box) does not significantly hasten completion of thrombin formation However there is no appreciable difference in the response to Ca + tpln (*3a* *3b*), which is complete (4 seconds c t) in one fourth to one half hour in both instances thus proving that the properties of the prothrombin itself are stable The lengthening of the clot lysis time by about one half day in *2b* *3b* hardly seems significant The conclusion favors the idea of a deterioration of a more essential thromboplastic factor than the protease impurity Our guess is that this refers to the thromboplastic phospholipid (cephalin like in character) *

III THROMBOPLASTIC ENZYME

Thromboplastic enzyme in relation to blood clotting and proteolytic phenomena

The Ferguson blood clotting theory[23] centers around a special thromboplastic role of small amounts of natural plasma or tissue proteolytic enzyme (*tryptase*[63] or

* It is possible that we were here encountering the effects of an unstable accessory factor [77] called *accelerator globulin* by Ware Seegers et al (see Addendum)

Other active tryptase preparations None of the other plasma fractions studied except the less purified *prothrombins* G and A (see table 1) and some commercial *thrombins* (e g table 20 TL_4) showed coagulant activity as well as protease effects Protease could not be detected (table 18) in the highly purified prothrombins (B—E) nor in certain thrombin preparations (table 20 TL_1) It is certain therefore that it must be regarded as an impurity Excepting cephalin (purified P lipid) all the *thromboplastic agents* (see *Reagents*) tested were found to contain a trace of tryptase (*cf* [40]) In view of this ubiquity of tryptase in all but the most highly purified plasma and tissue products it is not surprising that we were able to demonstrate both the lytic and thromboplastic (weak) effects of this protease in crystalline *serum albumin* preparations (Harvard Laboratory[9a]—see *Reagents*) of both human (H S A) and bovine (B S A) origin

T BLE 12 *P thr mbin Act ation T sts Effects of Aging (3 w ks)*

T 10 c l t g 4 c PRO C (0 4^cc) a d ted act i 0.5 cc M/10 CaCl₂ 0 5 t;l B (0 5^cc) borat buff pH = 7 PRO C 2 same as 2b b t 24 hou ld r (k pt ce hest)
C t (sec) at room t mp (1 2 C) f 0 5 B F (0.5^cc) + 0 5 c T

T	Dat	Act at	I cubati P od room t mperat											Cl t l s (3 C)
			¼ hr	½ h	1 h	2 h	4 hr	4 h	2 dy	4 dy	7 dy	11 dy	21 dy	
1a	1 14-47	(b ff ly)	610"	55	360"	210	145	8	36	26	12	½	½	0 (7 da)
1b	2 6-47	"	2 60"	2660"	2190"	2000	1500"	4 8	350"	245	130"	39"	15	0 (10 day)
2a	1 14-47	Ca o ly	16	133	8	45	31	13	9"	5½	4	4	—	2 day
2b	2 6-47		33	210	178	150	88	23	20"	10"	6	4	4	2½ da
2	2 -47		126	119"	105	9	60"	38	3	18	6	4	4	½ d y
3a	1 14 4	C + tpl	4	4	4	4	4	4	4	4	4	—	—	2 d
3b	2 6-47		5	4	4	4	4	4	4	4	4	4	4	2½ d y
4	1 16-4	C + trypt *D*	4	6	8	11	—	—	—	—	—	—	—	2 h

T yptase pt co t ll d b t 39 C (l f f t f see t bl 18)

Dog plasma tryptase Our current preparations of high potency plasma tryptase are not yet perfected (see *Reagents*) but the preliminary data are confirmatory of those on other tryptase preparations with a few important additions

Table 16 shows some prothrombin (PRO G) activation data using dog plasma tryptase (trypt D) with and without trypsin inhibitor and other activators

In T_2 1 500 tryptase slightly improves the weak clotting in citrate (*cf* T_1) but the most significant point is the virtual absence of activation in 1 to 2 days in both series The 95 per cent (approximately) unactivated prothrombin at the end of a 17 day period was completely converted to 4 seconds c t after 1 hr incubation with Ca + tpln *D* (see Footnote * table 16 A) (Fibrinolysis was advanced but not quite complete in T_2 series on the 7th day (37 C) whereas the control (T_1) series were negative)

The thromboplastic action of the tryptase is seen in T_4 and T_5 as compared with the T_3 control (Ca only) When thromboplastin *plus* Ca are the activators (T_9) the tryptase is hardly necessary but it does reduce the total activation time from 2 to 1 hr (T_{10})

Other data on streptokinase activated human fibrinogen (H Fb) dog $(NH_4)_2SO_4$ pptd fibrinogen (D fb)—see *Reagents*—are essentially similar

Thromboplastic (and thrombic) actions of III 3 and effects of trypsin inhibitors (see *Reagents*) Table 14 modified (by inclusion of (1) thrombin percentages and (2) clot lysis tests) from data in the above cited publication [34] shows a definite thromboplastic action of the protease contained in the Harvard human *plasma fraction* III 3 [9a]

The c t data of the thrombin dilution series in section B enable us to express the degree of activation in relative thrombin percentages (v p 1134) Those for the 1 hr incubation are included in section A Note

1 The 100 per cent activation by Ca + *tpln* (T_1) was actually obtained in ¼ hr and remained stable for a week or more

2 Ca alone (T_2), gave only 1 per cent in 1 hr

3 Ca + enzyme (T_3) increased this to 25 per cent a considerable thromboplastic effect

4 III 3 is not an ideal tryptase preparation because it has some thrombic action of its own but the control (T_6) shows this to be only 1 per cent quite unable therefore to account for the above effect

5 Pancreatic trypsin inhibitor (T_4) reduces the thrombin formation to 10 per cent

6 Soybean trypsin inhibitor (T_5) diminishes it to less than 1 per cent completing inhibiting the thromboplastic enzyme The question of an additional direct antithromboplastic action of S B I was raised on p 1142 (and see table 8) and is fully answered below

Nomenclature Our nomenclature [7] of the fibrinolytic and thromboplastic plasma protease as tryptase (f [59]) is tentative and based on the practical consideration of many similarities (despite differences in origin etc) to pancreatic trypsin There is no current agreement on nomenclature [8] however and there are certainly several plasma proteases e g cathepsins [43] and possibly papain like enzyme The coincidence of thrombic and proteolytic actions in III 3 might suggest a papainase (cf [32]) were it not for the fact that our other tryptase preparations lack the coagulant effect

Modes of action of trypsin inhibitors

1 *Antitryptic effect* These known pancreatic *trypsin* inhibitors were shown to inhibit plasma *tryptase* in fibrinogenolytic and fibrinolytic tests [33]

2 *Antithromboplastic effect* The later tests of T_5 series (table 14) suggest that S B I antagonizes not only the protease but also the natural thromboplastic (phospholipid) factor which continues to operate in the control (T_1) There is no good evidence for this in the case of T I The control tests (without added enzyme) in table 8 were made with the same prothrombin (PRO G) as the table 14 series They clearly show inhibition of prothrombin activation by S B I both (T_5) with Ca alone and more significantly (T_8) with Ca + tpln The T I shows negligible effects of this sort

It is concluded therefore that the *soybean* (not the *pancreatic*) trypsin inhibitor has additional direct antithromboplastic (? anticephalin) effects somewhat like the first phase action of heparin (table 9)

3 *Second phase controls* The negligible effects of trypsin inhibitors in the second phase are proved in the data of table 8 C An additional possibility that S B I (under certain circumstances) might serve as a co-factor for the antithrombic effects of heparin was also ruled out by some tests noted in table 15 Observe the slight immediate antithrombic effect of 25 Toronto units per cc (final concentration) of heparin on the thrombin (0 5 per cent h g) tested (in which there is no reason to suspect the presence of any co factor) The S B I had no antithrombic act on (a) immediately or (b) after 1 hr whether tested alone or with heparin

tivators This difficult goal is obviously not fully achieved by Seegers methods of purification Nevertheless some highly significant facts emerge from a critical consideration of certain differences in behavior of individual prothrombin preparations particularly when the preparations act in the presence of thromboplastic enzyme

Limitations of thromboplastic action of trypsin The differences between the data of figure 3 and table 17 are especially interesting

A Data for figure 3 were obtained on an old prothrombin preparation (PRO C) Thrombin percentages (100 per cent = complete activation) were obtained from a thrombin dilution series plotted as 1000 − c t (sec) in the dotted line and used to construct the activation curves I–IV The 5 cc volume thrombic mixtures all contained 4 cc PRO C (0 3 per cent) + 0 25 cc M/10 CaCl with the following additions (I) 0 75 cc buffer only (II) 0 25 cc buffer + 0 5 cc trypsin (40-unit/cc) (III) 0 5 cc buffer + 0 25 cc tpln *A* (0 25 per cent) (IV) 0 25 cc tpln + 0 5 cc trypsin Note the results (I) Ca (alone) produces slow activation complete in 50 hours (II) Ca + tryp is not significantly better (III) Ca + tpln is adequate but rather slow in the later stages thus extending the complete activation to about 24 hrs (IV) Ca + tpln + tryp is significantly best and completes over 90 per cent of the activation in less than 1 hour although here again the penultimate stages are still rather slow and require up to 16 or 18 hours for 100 per cent completion

Cl t lysis was followed for 4 days only in hich time it was absent in I incomplete in III and complete (24–48 hours) in II and IV Other te ts f iled to show any protease in PRO C (table 18)

B Table 17 also gives percentage data computed from a thrombin dilution series It gives a very good idea of the progress of the activation under various activator conditions Omitted from the table are (1) *Control tests* with Ca + tpln which show the fibrinogen to be prothrombin (and thrombin) free (2) Tests with PRO D and (a) buffer only and (b) + tpln only which show only negligible traces of thrombin already present in the prothrombin solution (Weak as they were these traces of clot resisted fibrinolysis for a week at 37 C)

Note the following results in sharp contrast to those of PRO C (fig 3) (T_1) a stronger tpln + Ca gave complete (100 per cent) activation in ½ hr while (T_2) a weaker tpln + Ca needs 48 hrs This is reduced by 4 u of trypsin to only 3 hrs but (T_5) tryp + Ca (without added tpln) is nearly as good except in the earlier stages (T_4) Ca alone is slower to start than Ca + tpln (T_2) but is complete in the same length of time (48 hrs) (T_6) Trypsin alone produces maximal activation in 6 hours but this amounts to only 20 per cent of the potential activity and even this rapidly falls off in the next 24 hrs Loss of thrombic potency after maximal activation is also seen in T_3 and T_5 Clearly the trypsin has prothrombinolytic and thrombinolytic powers

Cl t lys s fails to reveal any trypt se n the prothrombin (T) but there is ev dently a trace in tpln C (T) The tryptic fibrinolysis is re d ly detect d (T T T_6)

The fibrinolytic potency of the added tryptase is marked in T_5 and evident in T_4 (by completion of lysis in a day less than in T_3). The same is true of T_{10} versus T_9.

Two reasons are suggested for the failure of the thromboplastic action to be as strikingly evident as the fibrinolytic effect: (1) Deterioration of thromboplastic (? P lipid) impurity as previously noted (table 12). (2) Prothrombinolysis in case of stronger protease T_5 as shown by the longer early c.t. values together with some thrombinolysis definitely shown in the late c.t. values. The failure to reach

TABLE 13.—*Thromboplastic Action of Plasma Tryptase Compared with Standard Trypsin* I

A. *Preparation of activators. Timing of fibrinogenolysis*

Clotting time test: 0.5 cc mixt. + 0.25 cc thrombin enzyme free (h.g. 1%) at 24 C after stated incubation periods at 39 C

	(cc) Mixture	¼ min	¼	½	¾	1	1½ hrs	(Incub. at 39 C for Lysis)
A	4.0 H F (1%) 0.4 strep (1%)	6	15″	45	85	+	∞	(c.t. at 24 C)
B	4.0 H F 0.4 tryp (20 u.)	6″	21	51	90	+	∞	( )
C	4.0 H F 0.4 buffer	heat defibrinated at 56C (3 min.) filtered through glass wool						

B. *Prothrombin activation tests*

T: 5 cc vol. containing 2 cc PRO. B (0.2%) *recalcified* (0.25 cc M/10 CaCl) and mixed with 2.5 cc of cited activators (A, B, C)

C.t. (sec.) at 24 C, pH = 7.7 (borate buffer) for 0.5 cc B.F. (0.5%) + 0.25 cc T after stated incubation period at room temperature

T	Activator	Incubation Period (room temperature): ¼ min	½ h	1 h	2 h	6 h	18 h	24 h	4 dy	7 dy	Clot lysis (37 C)
		seconds									
1	Ca only	268	228	195	163	110	72	55	26	13	0 (7 day)
2	mixt. A	307	247	207	172	98	68	55	28	12	0 (7 day)
3	B	324	132	86	62	45	36	28	20	12	18 hr
4	C	335	58	40	27	13	8½	7	5	5	18 hr

less than a 13 second c.t. is especially significant. With the weaker tryptase, however, the first of these two reasons must be invoked. The similarity to the trypsin data of figure 3 in the section to follow is noteworthy.

Mode of action of thromboplastic enzyme. We have already (p. 1146) mentioned our theory that trypsin (experimentally) or tryptase (naturally) are only *accessory* to the basic mechanisms of prothrombin activation by calcium ions and thromboplastic phospholipid (cephalin). Tryptase, therefore, can be expected to work *only* in the presence of adequate amounts of the true activators. In the original presentation of the thromboplastic enzyme theory[3] it was commented that the final answer must await the preparation of prothrombin entirely free from all traces of its ac

plastin preparations are usually unsuitable The protease may be assayed in fibrinolytic units by our method A (p 1156) [1] using standard trypsin dilutions (1 mgm — 100 units) and an enzyme free thrombin (e g 1 per cent h g —see *Reagents*) to clot the fibrinogen

By this test significantly all Seegers highly purified prothrombins except PRO F (table 18) are quite free from tryptase This is definitely not the case however with (a) partially purified prothrombins (G A) nor (b) with most of the currently available *thrombin* preparations (table 20)

TABLE 14—*Thromboplastic Action of Plasma Tryptase II Human Plasma Fraction III 3 (Harvard) Effects of Trypsin Inhibitors*

A *Prothrombin activation data*

C t (sec) at 24 C pH = 7 7 (borate buff) 0 5 cc B F (0 5%) + 0 25 cc T

T 5 cc vol containing 2 cc PRO G (0 4%) 0 25 cc M/10 $CaCl_2$ 2 cc III 3 (0 1%) 0 25 cc tpln *B* (0 75%) 0 75 cc trypsin inhibitor (from pancreas T I 0 2% from soybean S B I 0 2%) where indicated

T	Activators	Inhibitor	¼ m	¼ h	½ hr	1 hr	~ 1 hr	1 dy	7 dy	Clot lysis (3 C)
			seconds					(seconds)		
1	Ca + tpln	0	78	4	4	4	100	4	4	2 day
2	Ca only	0	218	167	133	87	1	13	4	2 day
3	Ca + III 3	0	77	20	14	10	25	8	5	7 hr
4	+	T I	71	61	39	25	10	9	6	4 day
5	+	S B I	75	92	100	108	<1	150	45	0 (7 dy)
6	Ca + III 3	0	78	—	—	80	1	87	—	17 hr

B *Thrombin dilution (percentage) data*

C t (sec) at 24 C 0 5 cc B F (0 5%) + 0 25 cc T_1 (3 day old) dilutions stated

R I T co ~	100	50	25	10	5	1	0 5
Clotting time in seconds	4	8	11	22	41	84	127

II *Test for thrombin* Provided that the prothrombin is free from protease (or possibly by ensuring this by the addition (with controls) of a little trypsin inhibitor) the addition to 0 5 cc of the test fibrinogen of 0 25 cc of *citrated* PRO is at least a qualitative test for the presence of even very minute traces of thrombin

III *Test for thromboplastic factor* (? cephalin ?? lipoprotein) Test I gives some indication of the immediately available thromboplastic factor but supplementing T_1 with 0 5 cc of 20-unit/cc *trypsin* (see *Reagents*) before the clot timing (T 0 25 cc + B F 0 5 cc at room temperature) test series will greatly speed up the activation (to minutes or an hour or two instead of days) if available thromboplastic factor is present On the other hand deficiency of this factor will be shown by the lack of difference between III and I (Ca alone) control

IV *Test for available calcium* This must be made in the absence of decalcifying

The *activation tests* (with tryptase) in tables 13 and 14 resemble those of table 17 while the data in table 16 are suggestive of those in figure 3 The significant differences in enzyme (protease) effects incidentally noted in a large but not always complete, set of studies on PRO S A B, C D E F and G, are collected in table 18 Without entering into a detailed analysis of the data it can be concluded with considerable probability, that the differences consist in the varying amount of (a) thromboplastic phospholipid factor and (b) calcium (especially note T_7 table 7 *versus* T table 16) available (1) immediately or (2) because of the actions of the proteolytic enzyme for the prothrombin activation Lack of tryptase is a simple explanation of the unduly long incubation period necessary for Ca + tpln to activate highly purified prothrombins if it can be assumed that the *ac*

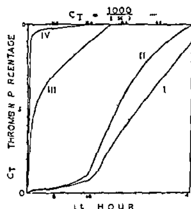


FIG 3 EFFECTS OF TRYPSIN ON THROMBIN FORMATION FROM PURIFIED PROTHROMBIN *apparently* CONTAINING INSUFFIECENT TRACE OF THROMBOPLASTIN (ref Ann N Y Acad Sci 49 486 1948)

Experimental data given in text Thrombin formation at successive incubation times (i t) computed from inverse clotting times (1000 — t (sec)) of thrombin (III) dilution series (dotted line) for the following mixtures I PRO C + Ca II PRO C + Ca + tryp III PRO C + Ca + tpln *A* IV PRO C + Ca + tpln *A* + tryp 25 C pH 7 7

celerator globulin (see Addendum) is adequate The data are all very clear in terms of the thermoboplastic enzyme theory

Suggested scheme for routine testing for trace impurities in prothrombin preparations The foregoing experimental analysis of the activation mechanisms suggests four simple routine tests which should be made on all prothrombin preparations

A 0 5 per cent prothrombin solution in our borate buffer (see *Reagents*) is recommended with the addition of 0 4 per cent (final concentration) of trisodium citrate (except in IV) Thrombic mixtures (T) 5 cc vol containing 2 5 cc PRO (0 5 per cent) + activators as follows (v infra) Clotting tests at room temperature on 0 5 cc prothrombin and enzyme free fibrinogen (e g 0 5 or 0 25 per cent B F the half strength being recommended for protease tests I)

I *Test for protease* (tryptase) by clot lysis at 37 C T_1 is activated by 0 25 cc M 10 $CaCl_2$ for suitable periods (e g 1 4 24 48 hrs) timing the clotting (at room temperature) and then incubating (37 C) for fibrinolysis If a suitable *enzyme free* thromboplastin (e g 1 1000 cephalin) is available it would greatly shorten the necessary incubation Owing to tryptase contaminants commercial thrombo

lytic properties Fibrinolysis or clot resolution occurs only when the clotting system is contaminated with some protease (e g tryptase) of plasma or tissue (e g *crude* thromboplastin) origin Tryptase impurity of the prothrombin itself

TABLE 1 —*Effects of Trypsin on Activat on of Prothr mbin in P esence of Various Activators*
Th mb d t See tabl 3

Percentage values of thrombin present computed from clotting time data after stated in cubation periods in tests on prothrombin free fibrinogen
T 5 cc vol containing 4 cc PRO D (0 2%) and stat d Activators *per cc* T

T	Act at rs			I cubat P riod room temperat e									Cl t l (3 C)
	C (M 10)	tpl C (0 1%)	tryp (40 u)	5 m	15 m	30 m	1 h	3 h	6 h	24 h	30 hr	48 h	
		mg											
1	0 05	0 15	0	40	80	100	100	100	—	—	—	—	not tested
2		0 05	0	1	5	9	20	30	35	70	80	100	6–7 da
3		0 05	4	35	42	60	75	100	50	3	1	—	3 day
4		0	0	—	±	±	+	1	4	40	80	100	0 (10 day)
5		0	4	6	25	35	60	100	80	4	1	—	3 day
6	0	0	4	—	+	<1	>1	9	20	2	+	—	3 day

TABLE 18 *Some Sig ficant D fferen in Enzym (Prot ase) Act tor Effects on Individual Prothrombins*
Compos t f th omb c m t es a d t m eq ed fo mpl a t t (I V)

Data c ted p	Ag t p 10 l f Th omb M t					I	II	III	IV	V
	P th	Tpl	C (M/10)	P te se (l t l)		Cal um al	C + n zym	C + tpl	C + tpln + zyme	tpln + zym
	P p (%)	Type (%)	ol	(a) dded	(b) PRO C					
		cc						*h*	*h*	*h*
	A( )4	4(0 1)0 5	0 5	tryp (40)	±(6 day)	4 hr	1h	2	—	—
T b 4	B(0 2)8	*A*(0 1)0 5		(40)	0 10 day)	4dy	1½h	2	—	—
8	4	—		(10)	0 7 da)	> dy	4dy	—	—	—
F g 3	C(0 3)8	4(0 2 0 5		(40)	0 4 day)	50h	4 h	24	16	—
T b 17	D 0 2 8	*C*(0 1)0 5	"	(40)	0(10 day)	48h	3h	48	3	—
T b 9	E(0 35)8	4(0 2)0 5		(10)	0 day)	8d	2hr	8	—	—
7	F(0 2)8	*A*(0 2)0 5		(40)	+(4 d y)	>4dy	½hr	1	—	½
1	G(0 4)4	*B* 0)0 5		tryptase	+(d y)	4dy	(½h)†		—	—
16				trypt se	+(2½d y)	11dy	11dy	2	1	—

P tease p th omb (l fied) teste by t m q d f r mpl te l t ly
† U t ll d t t 39 C

is ruled out in nearly all the highly purified products (table 18) studied but is shown in those that are only partially purified (esp G A) We are not yet fully able to account for the fact that the protease impurity always shows up best in *recalcified* thrombic mixtures McDonald and Kunitz[49] have shown the importance

anticoagulant Spontaneous activation and especially the acceleration of this by added thromboplastin (alone) is indicative of available calcium The most rapid and satisfactory test however is to use (T_3) a thrombic mixture containing PRO

TABLE 15 —*Lack of Antithrombic (second phase) Effects of Crystalline Soybean Trypsin Inhibitor with and without Heparin*

C t (sec) for 0 5 B F (0 5%) + 0 5 thrombic mixtures (I–IV) consisting of 0 25 cc h g (0 5%) + 0 125 cc each of inhibitors noted Tests made immediately (15 sec) and repeated after thrombic mixtures had stood at room temp (22 C) for 1 hr

M t e	I	II	III	IV
Inh b tor	none	S B I (0 5%)	hep (100 u t)	S B I + hep
15 sec incub	15	15″	37″	37
1 hr incub	15″	15	35″	35

TABLE 16 —*Effects of High Potency Plasma T yptase on Aged Prothrombin Activation and Inhibition (by Crystalline Pancreatic Trypsin inhibitor)*

4 *t t d t* E pts started 2 11–47 (f tables 8 d 12)

T 5 cc 1 t n ng 2 cc PRO G (0 4%) *t ated* (0 4%) and Activator (0 cc) and Inh b to (0 5 cc) c t d T ypt (trypt D) used n (fi al) diluti s n ted F l conc (mg/c T) f c trate = 160 f tryps nh b t T I = 0 Th omboplastin (tpl *B*) 0 75% ak d after 4 days in i e b

C t m (m) o c ( ) at room temp (2? ±2 C) f 0 2 cc T + 0 5 c B F (0 5%)

T	A t v to	T I	In bat on Period om t mpe t ¼ m	½ h	¾ h	1 h	2 h	4 hr	1 dy	2 dy	4 dy	8 dy	11 dy	17 dy	Clot lysis (37 C)
1	0	0	76m	6m	m	78m	79m	7 m	51m	49m	20m	7m	115	39	0 (10 d y)
2	trypt (1 500)	0	48m	4 m	46m	43m	4 m	44m	43m	41m	18m	5m	90	35	7 d y
3	C only	0	204	180	149	12	86	48	25	20	13	8	4	4	2½ day
4	Ca + t ypt (1 500)	0	183	136	117	83	54	39	22	19	9	5	4	4	1½ d v
5	Ca + trypt (1 20)	0	198	403	360	34	238	180	19	13	16	48	59	74	50 m n
6	Ca + trvpt (1 20)	0 5	184	156	135	111	74	54	27	6	19	11	7	5	3 d y
7	Ca + trypt (1 500)	0 5	180	14	14	1 1	101	86	36	30	25	13	7	4	4 d v
8	Ca only	0 5	188	17	159	14	126	112	43	35	8	20	7	4	4 d y
9	Ca + tpl	0	100	14		5	4	4	4	4	4	4	4	4	2½ d y
10	Ca + tpln + t ypt (1 500)	0	88	13	6	4	4	4	4	4	4	4	4	5	1½ d y

Afte 1 hr b w th Ca + tpl *D* 17th day T and T w both br ught to 4 c t val

(see T_1) without calcium but *with* 0 25 cc thromboplastin (Ca free) *and* 0 5 cc 20-unit/cc trypsin (Ca free)

By the use of these (or similar) tests we secured the tentative data on trace impurities of the various prothrombin preparations noted in table 1

V TEST FOR ACCELERATOR GLOBULIN (see Addendum)

Proteolytic phenomena in relation to coagulation processes

I *Fibrinolysis* It is clearly proven by the data of these and earlier[30] studies that thrombin itself or its prothrombin precursor are devoid of ordinary proteo

lytic properties Fibrinolysis or clot resolution occurs only when the clotting system is contaminated with some protease (e g tryptase) of plasma or tissue (e g *crude* thromboplastin) origin Tryptase impurity of the prothrombin itself

Tᴀʙʟᴇ 17 —*Effects of Trypsin on Activation of Prothrombin in Presence of Various Activators*
Th mb d l t S tabl 3

Percentage values of thrombin present computed from clotting time data after stated incubation periods in tests on prothrombin free fibrinogen
T 5 cc vol containing 4 cc PRO D (0 2%) and stated Activators *per cc* T

T	A t at			I cubat o P od room tempe ture									Cl t ly is (37 C)
	C (M/10)	tpl C (0 1%)	tryp (40)	5 m	15 m	30 m	1 h	3 h	6 h	24 h	30 hr	48 h	
		mg											
1	0 05	0 15	0	40	80	100	100	100	—	—	—	—	not tested
2		0 05	0	1	5	9	20	30	35	70	80	100	6–7 day
3		0 05	4	35	42	60	75	*100*	50	3	1	—	3 day
4		0	0	—	±	±	+	1	4	40	80	100	0 (10 dav)
5		0	4	6	25	35	60	*100*	80	4	1	—	3 day
6	0	0	4	—	+	<1	>1	8	*20*	2	+	—	3 day

Tᴀ ʟᴇ 18 *S me Significant D ff r n es in En ym (P ote e) A ti ator Eff cts on Ind id al Prothrombins*
Compo t f th omb e m t s a d t m eq ed f *c mpl t* act at n (I V)

D ta c t d p	Agents *pe 10* *l* f Th mb M t e					I	II	III	IV	V
	P othr	T l	C (M /10)	P ot (1 t ly)		C l m	C + zym	C + tpl	C + tpln + en zym	tpl + zyme
	P ep (*m*)	Typ (*m*)	1	( ) dd d	(b) PRO C					
	cc							*h*	*h*	*l*
	A( )4	*A*(0 1)0 5	0 5	tryp (40)	±(6 d y)	48h	1h	2	—	—
T b 4	B(0 2)8	*A*(0 1)0 5		(40)	0(10 d y)	4dy	1½hr	2	—	—
8	4	—		(10)	0(7 d y)	>7dy	4dy	—	—	—
F g 3	C(0 3)8	*A*(0 25)0 5		(40)	0(4 d y)	50h	48hr	24	16	—
T b 17	D(0 2)8	*C*(0 1)0 5		(40)	0(10 d y)	48h	3h	48	3	—
T b 9	E(0 35)8	*A*(0 2)0 5		(10)	0(d y)	8dy	2hr	8	—	—
	F(0 2)8	*A*(0 25)0 5		(40)	+(4 d y)	>4dy	½h	1	—	½
12	G(0 4)4	*B*(0 5)0 5		trypt	+(2 d y)	4dy	(h) †	½	—	—
16				t yptase	+(2½d y)	11dy	11dy	2	1	—

P ot p th omb n (al fi d) t t d by t m q d f mpl t l t ly
† U o t oll d t st at 39 C

is ruled out in nearly all the highly purified products (table 18) studied but is shown in those that are only partially purified (esp G A) We are not yet fully able to account for the fact that the protease impurity always shows up best in *recalcified* thrombic mixtures McDonald and Kunitz[49] have shown the importance

of calcium in the formation of trypsin from crystallized trypsinogen (pancreatic) but our experiments to date have not definitely proved whether this kind of action is involved in the plasma tryptase system

Clot retraction is evidently a preliminary stage of fibrinolysis in our in vitro tests where it is not at all uncommon to observe a very weak protease cause retraction of the clot without complete fibrinolysis (e g T_c table 11) In ordinary blood clot retraction platelets and other cellular elements[53] may very well contribute proteolytic enzyme(s) [5] We have found tryptase in platelets by means of the fibrinogenolytic test It is not improbable that colloidal syneresis of the nature of elastic retraction due to micellar rearrangement may also be involved in clot retraction but a partial fibrinolysis could be an essential initiating force

In a somewhat analogous manner a colloidal phenomenon (coacervation Mommaerts[51]) characterized by interlacing of the elongated (filamentous) fibrinogen molecul s and micelles may be the funda

TABLE 19 —*Prothrombinolysis by Plasma Tryptase*

PT = 10 cc PRO H (0 1%) *citrated* (0 4%) + 0 625 cc Tryptase *D* 2 cc samples of PT removed at age cited and activated (T) by 0 125 cc each of M/10 $CaCl_2$ tpln *D* and borate buffer C t (sec) 0 5 cc B F (0 5%) + 0 25 cc T Temp 25 C pH 7 7

T	Age Pt	½ m	5 m	20 m	1 hr	hr	6 hr (t)	Clot lys
		(d)						
	min							
1	½	136	6	4	3½	3½	3½	12–15 m (37 C) 1 hr (25 C)
2	15	292	35	7	5½	5	4	
3	30	435	72	22	13	10	6	
4	60	612	247	123	77	48	16	

* Alternate tubes tested at the respective temperatures

mental explanation of their aggregation to form fibrin but here again a specific causative agent is necessary and this is the function of thrombin Thrombin is not proteolytic (v supra) but the proteolytic enzyme papain (see p 1148) which can also produce true fibrin also has fibrinolytic powers [11]

II *Fibrinogenolysis* As we have previously shown lysis of fibrinogen [0] even better than fibrinolysis [1 4] is an excellent quantitative method for assaying trypsin like enzymes with a sensitivity of the order of 1 1 000 000 The two tests which we term method B and method A respectively may be adapted for the study of natural proteases related to the blood clotting system which study is being further prosecuted in our laboratories *

It is significant that small amounts of contaminating plasma proteases do not greatly interfere with the clotting system In part this may be due to the wide range of fibrinogen concentrations that give optimal clotting (table 2) In certain situations however such as the noncoagulability of cadaver[61] and menstrual blood [45 65] a simple explanation is forthcoming on the basis of the fibrinogenolytic action of larger amounts of tryptase *with* or *without* thrombic clotting

* More recent experiments emphasize practical difficulties due to differences in the shape of the lysis curves (plotted against incubation time)

III *Prothrombinolysis* is the other factor to be considered in poorly clotting blood plasma (e g after citrated storage) or exudates Prothrombinolysis may precede or accompany fibrinogenolysis A simple test with thrombin will quickly show up any fibrinogen present If this is negative fibrinogen should be added[69, 6] but otherwise this is unnecessary before going on to a test for prothrombin by the addition of optimal amounts of calcium and thromboplastin (e g the Quick test[59])

We have long been aware of the prothrombinolytic action of trypsin[30] and Seegers and Loomis[80] have recently noted this with tryptase (fibrinolytic enzyme) Some evidence of prothrombinolysis is observed with the high potency tryptase in the activation tests (T_6) of table 16 but the phenomenon is best seen on incubating prothrombin solution with tryptase and testing samples at intervals for activation with Ca + tpln (table 19) The data of table 19 clearly show the progressive weakening of the prothrombin by the added tryptase

TABLE 20 —*Thrombinolytic Action of High Potency Plasma Tryptase*

TL thrombin lysis mixt containing 1 cc thrombin (type specified—see Reagents) 1 cc trypt D and buffer (to 2.5 cc vol) Incub (of TL) and clotting tests at room temp C t (sec) 0 25 TL + 0 5 B F (0 25%) Tryptase used in 1 3 5 buffer only (controls) in 2 4 6

TL	Thrombin (type)	TL-incubation period (room temperature)								Clot lysis
		½ m	1 h	2 h	4 h	8 h	1 dy	2 dy	4 d	
		seconds								
1	Tu (1%)	10	10	10½	12	13	19	40	—	6–10 min (22 C)
2		10	10	10	10	10	10	10	11	>7 day (22 C)
3	h g (1%)	10	10½	11	12	13	17	36	—	6–10 min (22 C)
4		10	10	10	10	10	10	10	11	0 (7 day 37 C)
5	T_{PD} (100 u)	6½	6½	6½	7	7	9	12	21	5–10 min (22 C)
6		6½	6½	6½	6½	6½	6½	6½	7	<7 day (37 C)

IV *Thrombinolysis* Trypsin (pancreatic) also causes thrombinolysis akin to the natural irreversible disappearance of thrombin in serum[39] We have noted however that this requires rather large amounts of trypsin[0] Several recent workers[60, 46] claim that thrombinolysis is not produced by tryptase In the light of the trypsin data (e g tables 4 T_4 7 T_8 T_6 T_7 9 T_3 but *cf* 13 T_4 with small amount of trypsin) we were rather surprised at these claims and at our similar negative tests on a number of occasions (e g table 14 T_3) Very recently we have obtained some high potency dog plasma tryptase (trypt *D*) (see *Reagents*) One of these preparations tested at 39 C (without control) in T_4 of table 12 was highly suggestive but convincing evidence of the thrombinolytic action even at room temperature was given by an even better tryptase preparation tested on half a dozen different thrombins including T_1 T_3 and T_9 of table 16 and the commercial thrombins shown in table 20 The perfect stability of the controls and the potency in clot lysis (and in fibrinogenolysis) of the tryptase are brought out in the data One incidental finding was that thrombinolysis could *not be followed beyond a* certain point because the tryptase persisting in the lytic mixture destroyed the

fibrinogen in the clotting test mixture more rapidly than the erstwhile potent thrombin could clot it A definite shortening of the fibrinolytic times in the tests prior to this i e while the thrombin though weakening was still present, could be ascribed to the same cause *Trypsin inhibitors* (T I and S B I) prevent the thrombinolytic effect

V *Lysis of accelerator globulin* (see Addendum) is another possibility

Summary

A number of high potency purified prothrombin preparations [61] in 0 2–0 5 per cent solution in borate buffer (pH = 7 7) maintain for days or weeks a stable thrombin forming ability whether (a) with buffer alone (b) with brain thromboplastin suspension (c) with CaCl Nevertheless they all contain a trace of thrombin and continue to activate spontaneously at a very slow rate Optimal addition of Ca salt somewhat accelerates this and usually leads to maximal (complete) thrombin formation in 2–11 days at room temperature Except in a few cases where ionized Ca^{++} is demonstrable thromboplastin alone is without effect but added *with* calcium it completes the activation in a matter of minutes or hours depending principally upon the concentration used

Experimental analysis of the activation process stresses the participation of (1) Ca ions (2) thromboplastic P lipid factor (cephalin) (3) plasma and tissue tryptase (proteolytic enzyme) Each of these factors is studied in detail with reference to mode of action optimal concentration side effects and relation to inhibitors

Inhibition of prothrombin activation may be considered under the following heads (1) decalcifying agents (e g oxalates citrates etc) which (a) depress Ca ionization and thus prevent thrombin formation and (b) *under special circumstances* reverse the process of activation (2) antithromboplastic (? anticephalin) agents (e g heparin and probably soybean trypsin inhibitor to some extent) (3) antitryptase agents (e g crystalline trypsin inhibitors from pancreas and soybean) which inhibit the thromboplastic enzyme (accessory factor) Excess Ca^{++} slows *rate* of thrombin formation

The evidence suggests that thrombin formation proceeds via an intermediary calcium prothrombin cephalin (thromboplastic phosphatide) complex or compound The amounts of (a) thromboplastic P lipid (cephalin or thromboplastin) and (b) Ca^{++} determine both the rate of activation and the final thrombin yield However the ultimate (ripe) thrombin owes none of its activity to the presence of any calcium or phospholipid

The three types of activator (Ca thromboplastin and thromboplastic enzyme) occur as trace impurities in prothrombin preparations but Seegers most purified materials are tryptase free Tryptase (and trypsin) are thromboplastic only in the presence of adequate calcium and phospholipid factors which may however be mobilized from protein combination including prothrombin In this way the two basic activators are in a sense catalyzed in their prothrombin activating reactions

The significance of proteolytic actions (fibrinolysis fibrinogenolysis prothrombinolysis and sometimes thrombinolysis) by natural tryptase of plasma and tissue

origin is investigated and discussed in relation to the broader asp cts of th blood coagulation problem

ADDENDUM

Since this paper was submitted for publication an entirely new aspect of the process of prothrombin activation has been opened up by the discovery of a new clotting factor variously designated but which may be referred to by the term *accelerator globulin* (Ware Seegers et al) Dr Seegers assures us that all the prothrombins here reported on contain an abundance of this factor (which is ex tremely potent) Further the strength of our prothrombin solutions and the experimental conditions noted make it improbable that the data herewith re ported will be invalidated by increasing knowledge of the new factor One possible exception is the change in activation properties in some of the prothrombins discussed

ACKNOWLEDGMENTS

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The human plasma fractions used in this work were prepared from blood collected by the American Red Cross under contract between Harvard University and the Office of Scientific Research and Development The figures are reprinted by permission of the original publishers cited

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THE EFFECT OF INTRAVENOUS INJECTION OF TRYPSIN INHIBITOR ON THE COAGULATION OF BLOOD

By H J TAGNON M D AND J P SOULIER M D

THE TRYPSIN inhibitor isolated from soya bean flour by Kunitz[1] has been shown to have anticoagulant activity in vitro [2] The present communication deals with the effects of the intravenous injection of this material into experimental animals The action on the clotting time the prothrombin time and the antitryptic activity of blood plasma and serum were studied

METHODS

Trypsin i h b tor A crude preparation was obtained by the method described previously [3] Dialysis of the final preparation for twenty four hours against saline in the i e box was found to be necessary for the removal of a toxic factor present in the undialyzed material Without dialysis small amounts (0 5–1 cc) of the material regularly killed the 5 pound rabbits in a very short time (30 to 60 seconds) with cardiac standstill in diastole It is possible that the toxi ity was due to the presence of potassium ions in the preparation

The final preparation was spun at 2500 rpm for 15 minutes in order to remove all insoluble material and the pH as adjusted to 7.4 by the addition of NaOH N/10 Enough material was prepared in one batch so that all experiments except one (in which the crystalline inhibitor was used) were carried out with the same batch of material It was kept frozen at −30 C and warmed up to 37°C shortly before the injection was given

The preparat on of inhibitor vas assayed against crystallin trypsin (obtained from the Plaut Research Laboratory Bloomfield N Y) by the method of Anson Twenty five milligrams of this trypsin preparation produced 0 1137 mg of tyrosin in 10 minutes at 25 C The addition of 0 2 cc of the inhibitor preparation used in this work to this quantity of trypsin reduced the production of tryosin from 0 1137 mg to 0.0108 mg

A small quantity of crystallin trypsin inhibitor recrystallized 3 times obtained by the method of Kunitz[1] was used in one single experiment as indicated below

Exper mental an mals 2 mongrel dogs and 3 rabbits received injections of the inh b tor preparation One additional rabbit was injected with the crystallin material The animals were anesthetized by the intravenous injection of from 25 to 35 mg of nembutal per kg Injections were mad into the jugular vein in th dogs and the ear vein in the rabbits Blood samples were obtained by syringe and needle from the other jugular vein in the dogs and from the carotid arteries in the r bb ts The blood samples were taken simultaneo ly with and without anticoagulant A mixture of 2 parts of potassium oxalate and 3 parts of ammonium oxalate in dry form was used as an anticoagulant Ten milligrams of the mixture was used for every 5 cc of blood The plasma was removed immed ately by centrifuging

The clotting time was stud ed on blood taken without anticoagulant by a modification[5] of the method of Lee and White [6]

The prothrombin time was measured on the plasma by the method of Quick [7]

The presence of an anticoagulant in the samples showing a prolongation of the clotting time was tested as described previously [8]

In the experim nts on rabb ts samples of plasma were tested for their antitryptic acti ity before and after the injection of trypsin inhibitor preparation One h lf cubic centimeter of oxalated plasma was mixed with one h lf cubic centimeter of a solution of crystallin trypsin and the proteol tic acti ity of

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the mixture measured on a hemoglobin substrate by incubation at 37 C for ½ hour according to the method of Anson [4] The crystallin trypsin was the same used for the assay of the inhibitor

In the experiments on dogs samples of blood serum were tested before and after the injection of trypsin inhibitor for antiproteolytic activity against the blood plasma enzyme As shown previously the trypsin inhibitor from soya bean is also inhibitor towards the blood plasma enzyme [2] This was done by measuring the rate of dissolution of 0 1 cc of fibrinogen solution by a chloroform plasma preparation (containing the active plasma proteolytic enzyme) in the presence of each sample of serum as described previously [2]

Results

Tables 1 and 2 show the overall results obtained in the experiments on two dogs and four rabbits

1 *Effect on clotting time* There was an immediate prolongation of the clotting time following the injection of the soya bean preparation in dogs as well as in rabbits This effect was transient lasting from forty minutes to one hour after the injection of respectively 8 cc per Kg and 5 cc per Kg into the 2 dogs and from 30 to 60 minutes in the 3 rabbits that were followed longer than one hour

Table 1 —*The Effect of the Intravenous Injection of Trypsin Inhibitor on Clotting Time Prothrombin Time and Fibrinolysis*

Dog #	Trypsin inhibitor (cc)	Clotting time at 37 Intervals						Prothrombin time Intervals						Time of lysis of fibrinogen in presence of serum from blood taken at stated intervals					
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 16 Kg	85	8	55	40	24	22	9	7	10	9 5	9 5	9 5	9 5	3	106	136	136	121	76
2 10 Kg	80	10	52	13	11			14	16		16½	16½		11	180*		180*	120	

* 0 1 cc fibrinogen + 0 1 cc serum + 0 4 cc chloroform plasma preparation

** 1 before injection 2 from 5–10 minutes after injection 3 from 10–30 minutes after injection 4 from 30–40 min after injection 5 from 46–60 min after injection 6 from 60–90 min after injection

The injection of the small available quantity of crystallin inhibitor into rabbit # 4 produced a small prolongation of the clotting time (table 2 exp 4)

2 *Effect on prothrombin time* This was prolonged following the injection into dogs and rabbits and remained prolonged for a longer period than the clotting time (tables 1 and 2)

3 *Effect on antiproteolytic activity* of blood serum and blood plasma In the 2 experiments on dogs a chloroform plasma preparation (containing the active plasma proteolytic enzyme) was mixed with serum from blood obtained before and after the intravenous injection of trypsin inhibitor and the mixture was tested for fibrinolytic activity on a solution of fibrinogen Table 1 shows that the time for complete fibrinolysis of the clot increased sharply in the presence of serum from blood obtained immediately after the injection of the inhibitor The time of fibrinolysis was still considerably prolonged at the end of the 2 experiments (table 1) Figure 1 presents the results of experiment 1 in graph form

In the 4 experiments on rabbits the amount of tyrosin produced by trypsin in

TABLE 2.—*The Effect of the Intravenous Injection of Trypsin Inhibitor on Clotting Time Prothrombin Time and Antitryptic Act ity of Plasma*

Rabb ts #	Type of pr pa ation	Cl tti g tim t 3 C m							Prothromb n tim sec							Qu tity of tyrosin prod ced by trypsi in pres of plasma (mg X 10⁻⁴)						
		1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1 2 kg 300	Soya bean prepar 17 cc	10	25			13	12	11	10	15			13	13	11	18 2	660			1980	1794	2238
2 2 kg 300	Soya bean prepar 30 cc.	1	0	24					13		15					828	249	369				
3 2 kg 300	Soya bean prepar 20 cc	11	27		13	11	7	8	17	23		20	16	21	23	974	468		381	345	927	
4 3 kg 100	Crystallin in hib 45 mg in 9 cc sa line	8	13		10	7	6	5	1-	23		16	16	15	13	1736	300		984	1182	1182	1112

1 before injection 2 5 minutes after injection 3 15 minutes 4 30 min 5 60 min 6 2 hours 7 3 hours

0 5 cc oxalated plasma + 0 5 cc solution of trypsin + 5 cc hemoglobin substrate Incubation ½ hour at 37

Solution of trypsin 30 mg in 10 cc of water in experiments 1 and 2.
20 10 cc 3 4

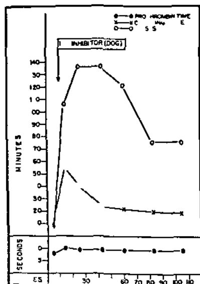


FIG 1 THE EFFECT OF INTRAVENOUS INJECTION OF SOYA BEAN PREPARATION ON ANTIPROTEOLYTIC ACTIVITY O BLOOD S RUM CLOTTING TIME AND PROTHROMBIN TIME

the presence of samples of plasma showed a sharp decrease with plasmas obtained after injection of the inhibitor (table 2) There was a gradual disappearance of the

increased antitryptic effect of plasma and normal or near normal values were obtained between 1 and 2 hours after the injection Figure 2 shows the results of experiment #4 (table 2) in graph form

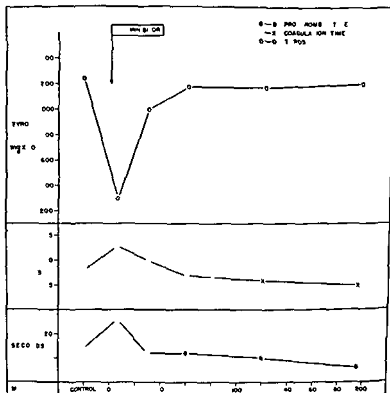


FIG 2 THE EFFECT OF INTRAVENOUS INJECTION OF CRYSTALLIN TRYPSIN INHIBITOR ON ANTITRYPTIC ACTIVITY OF BLOOD PLASMA CLOTTING TIME AND PROTHROMBIN TIME

TABLE 3 —*Protamine Titration of Plasma after Injection of Soya Bean Preparation*

One tenth cc of citrated plasma obtained from blood showing a prolonged clotting time following the injection of soya bean preparation was added to varying quantities of protamine The plasma was recalcified and the clotting time noted

P t m	Clott g t me t 37 C	
	D g *2	D g *3
0	8	10
0 01	30+	30+
0 004	30	10
0 001	11	9
0 0005	11	9
0 0001	11	7

In order to rule out the presence of heparin in blood showing a prolonged clotting time following the intravenous injection of soya bean preparation a protamin titration was carried out on the plasma from such blood in the 2 experiments on dogs Table 3 gives a typical example of such a titration in dog # [illegible] The results

show that there was no significant shortening of the clotting time of the recalcified plasma by the addition of various quantities of protamin

Discussion

The data show that a soya bean preparation containing the trypsin inhibitor produced the following effects when injected intravenously into 2 dogs and 3 rabbits it prolonged the clotting time of the blood the prothrombin time of the blood plasma and increased the antiproteolytic activity of the blood serum or plasma The same effects were obtained in one experiment on a rabbit in which a highly purified three times recrystallized trypsin inhibitor preparation from soya bean was used

There was some parallelism among the effects of the injection on the clotting time the prothrombin time and the antiproteolytic activity of the plasma This parallelism was most apparent in the experiments on rabbits because they were conducted for a longer time than those on dogs The coincidence of the three actions appears clearly in figure 2

The one single experiment carried out with the purified material gave results essentially similar with those obtained with the cruder material

If one considers the fact that the trypsin inhibitor produced all these effects when added to blood *in vitro* it seems that the data reported here might constitute evidence that the trypsin inhibitor prolonged the clotting time *in vivo* by the same mechanism by which it prolongs it *in vitro* and not indirectly by provoking the organism to release an anticoagulant in the blood stream This is further confirmed by the fact that the blood showing a prolonged clotting time following the injection of the trypsin inhibitor did not contain any heparin in the two experiments in which a protamin titration was carried out It is well known that the prolongation of the clotting time following the intravenous injection of peptone or of antigen in sensitized animals is due to the appearance of heparin in the blood of such animals

Three different tyrpsin inhibitors so far have been reported as having anticlotting properties the trypsin inhibitor from pancreas [10] from blood serum [10] and that from soya bean These three substances differ chemically and their similar action on the blood coagulation mechanism parallels their similar action on trypsin It is to be noted that the trypsin inhibitor from soya bean and from pancreas also inhibits the proteolytic enzyme of blood plasma[11 1] The exact role of the plasma enzyme in blood coagulation is unknown a recent communication presents evidence that the enzyme has no clotting activity by the usual tests [1] It is nevertheless interesting to note that these substances which inhibit the proteolytic action of the enzyme also are anticoagulant agents

The practical use of the trypsin inhibitor from soya bean for anticoagulant therapy must await further study The material does not appear to be toxic and could conceivably be used in purified form for prolonging the clotting time *in vivo* when such action is desired However it is quite possible that the inhibitor is antigenic and this point should be clarified before an attempt at practical application is made

Summary

1 The intravenous injection of a soya bean preparation containing a trypsin inhibitor into 2 dogs and 3 rabbits produced the following effects prolongation of the clotting time and of the prothrombin time and increase in the antiproteolytic activity of the blood plasma or serum

2 Identical results were obtained in one experiment in which a crystallin trypsin inhibitor from soya bean was used

3 The significance of these results is briefly discussed

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FIBRINOLYSIS ITS MECHANISM AND SIGNIFICANCE

By R G Macfarlane M D and Rosemary Biggs M B Ph D

Introduction

THE BIOLOGIC processes that produce a gross physical change have always attracted much interest The transformation of fluid blood to solid clot for instance has been the subject of experiment and discussion the amount of which seems disproportionate to its fundamental physiologic importance There is however a converse to this process of coagulation which though equally obvious in its result has been comparatively neglected possibly because in its extreme form it occurs only rarely and with apparent irregularity This is the phenomenon of *fibrinolysis or dissolution of blood clot that though normally slow may occur* with great rapidity under certain conditions Though there has been relatively little work on this subject recent developments have made it possible to appreciate part of the mechanism that underlies it and to realize that factors are involved which are of basic importance It is apparent also that the process of fibrinolysis is linked with other subjects previously without obvious association, and this paper though it cannot claim to be a complete review is presented as an attempt to assemble relevant information and to relate it to some observations of our own

Definition

For the purpose of this communication fibrinolysis is taken to mean the aseptic dissolution of fibrin brought about by the direct action of a mechanism existing in normal blood Usually dissolution may take days or weeks to complete but may be so accelerated as a result of natural changes occurring in the living subject or of experimental procedures as to occur within a few hours or minutes This increased activity is the main subject dealt with and though a particular set of factors have been studied as being apparently those mainly responsible it is recognized that others may be involved

The Recognition of Fibrinolysis

It is an ancient observation that circumstances may modify the permanence or stability of blood clots Zimmermann (1846) found that ox fibrin suspended in salt solution remained intact for up to 10 days while fibrin from human blood obtained by wet cupping dissolved in 12–24 hours He refers to earlier observations of a similar nature by Denis (1838) Green (1887) also studied the disappearance of fibrin in saline which occurred without obvious bacterial action and found that once it had disintegrated fibrin could not be made to clot again by adding thrombin Similar observations on the blood of dogs subjected to hemorrhage were made by Dastre (1893 1894 a & b 1895) who apparently the first to use the term fibrinolysis came to the conclusion that fibrin is digested and not dissolved though he did not favor the idea of a lytic enzyme in blood Rulot (1904) believed

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that leukocytes were concerned in the digestion since an increase in their concentration hastened the reaction In 1905 Nolf began a series of experiments in which he induced fibrinolytic activity in dogs by various procedures including hepatectomy and peptone shock He regarded this work as demonstrating the participation of a proteolytic enzyme in the process of blood coagulation to which he believed fibrinolysis was a logical if unusual sequel Morawitz (1906) studied the familiar phenomenon of the fluidity of the blood in cases of sudden death He observed that blood from such cases contained itself no fibrinogen and was capable of destroying the fibrinogen and fibrin of normal blood Hirose (1934) supported Nolf in regarding the lysis of fibrin as the result of coagulation and also considered that it was the cause of clot retraction

In 1937 Yudin stimulated a considerable new interest in fibrinolysis by its practical if rather macabre application to a blood transfusion service in Russia It was the practice to use the blood from fresh corpses and for obvious reasons the most suitable donors were the victims of accidental or sudden death rather than those who had died from a long drawn out disease This selection of subjects proved to have another advantage for in these cases of sudden death the blood removed from the body though it clotted in the usual way reliquified within a few hours and could be used for transfusion without anticoagulant Since it appeared that shock might be a relevant factor in Yudin s subjects Macfarlane (1937) investigated the occurrence of fibrinolysis in living patients who had undergone surgical operation There was an occasional instance of rapid lysis of whole clotted blood but this was a rare and apparently capricious occurrence It was found however that fibrin prepared from diluted plasma disappeared rapidly in a high proportion of post operation cases though similar preparations made before operation were stable for weeks an observation that was soon confirmed by Imperati (1937) and later by Kaulla (1947) Mole (1943) and Wexler and Ellis (1944) confirmed the occurrence of fibrinolytic activity in the blood of cases of sudden death Though Smith and Smith (1945) and Wilson and Munnell (1946) have reported that blood taken during menstruation shows fibrinolytic activity which also occurs according to the latter workers in hypertension and eclampsia the technic used makes it difficult to assess the significance of these observations Tagnon Levenson Davidson and Taylor (1946) *have observed fibrinolysis in human cases of severe burns and in one* case of fatal narcotic poisoning and have been able to induce similar activity in the blood of dogs by severe hemorrhage They quote an observation by Ham that fibrinolysis sometimes follows the intravenous administration of typhoid vaccine in human beings

The Mechanism of Fibrinolysis

From these scattered observations it is clear that rapid dissolution of fibrin occurs following a variety of disturbances to the living organism which may be due to an acceleration of the normal slower process or to some other agency The fact that lysed fibrin is irreversibly altered coupled with the observations of Morawitz (1906) and Mole (1943) makes it probable that fibrinolysis is the result of proteolytic digestion and it would be logical to trace the recognition of a proteolytic

enzyme in normal blood Since fibrin in sterile whole blood usually remains intact for some days or weeks it follows that such an enzyme if it exists must be under normal conditions relatively inert though capable of activation

Activation by Chloroform

In 1889 Denys and Marbaix found that a thermo-labile proteolytic agent developed in serum after the addition of chloroform ether or thymol apparently the first observation of a phenomenon since much studied Delezene and Pozerski (1903) investigated this reaction further showing that serum after admixture with chloroform developed the power of digesting gelatin and casein an activity that was inhibited by the addition of untreated serum They came to the conclusion that a proteolytic enzyme present in the serum was normally inhibited by some other substance which was removed or destroyed by chloroform a suggestion that could be related to previous observations by Hildebrandt (1893) and Hahn (1897) that normal serum is capable of inhibiting proteolysis by trypsin

Jobling and Peterson (1914) came to the conclusion that the antitrypsin of plasma was soluble in lipoid solvents a view which has recently been revived by Ungar (1945) Dale and Walpole (1916) and Yamakawa (1918) confirmed the destruction of the inhibitor by chloroform and Opie Barker and Dochez (1911) found that the administration of chloroform by stomach tube to dogs resulted in protein digestion occurring in serum samples and apparent destruction of fibrinogen Nolf (1921 a & b 1922) found that proteolytic activity accompanied by fibrinolysis could be generated in mammalia and bird plasma by the action of chloroform The action has been extensively investigated by Tagnon (1942) and Tagnon Davidson and Taylor (1942) mainly from the point of view of its relation to blood coagulation They found that the globulin fraction from chloroform serum was strongly fibrinolytic and might destroy fibrinogen before clotting could occur

Fractionation of Plasma

The fact that separation of the plasma proteins may result in proteolytic activity has also long been recognized Hedin (1904b) fractionated ox serum by ammonium sulphate precipitation and found that the globulin contained a proteolytic enzyme that was inhibited by a thermo labile factor associated with the albumen fraction Opie and Barker (1907) confirmed these findings and observed the similarity between the globulin enzyme and leuko protease More recently Feissly (1942) and Macfarlane and Pilling (1946a) have studied the fibrinolytic activity of globulin fractions separated by acid precipitation Taylor et al (1945) who have studied plasma fractions provided by Professor Cohn and Dr Edsall have observed spontaneous proteolytic activity associated with fractions I and III–2 It is probable that fractionation separates an enzyme inhibitor complex analogous to the dissociable tyrpsin antitrypsin compound studied by Hussey and Northrop (1923) The relationship in the latter case obeys the mass action laws and dissociation may occur following simple dilution of the complex a fact which is also relevant to the plasma enzyme and its inhibitor

Separation of the enzyme and inhibitor has also been accomplished by the use

of trichloracetic acid Schmitz (1937) found that the enzyme was precipitated by this agent while the inhibitor remained in the supernatant fluid He came to the conclusion that fibrinolysis was due to the adsorption of the enzyme together with a kinase onto fibrin Iyengar and Sehra (1942) have also utilized trichloracetic acid in the estimation of enzymatic activity of plasma in a variety of conditions Mole (1943) working with blood from cases of sudden death found that the rapid fibrinolysis was due to the action of an enzyme precipitable by trichloracetic acid This enzyme resembled trypsin in some respects but was shown to be significantly different in others

Separation of the enzyme has also been accomplished by adsorption onto fibrin Barker (1908) studied the proteolytic enzyme associated with fibrin and Rosenmann (1922 1936 1937) has used lysed fibrin as the source of an enzyme preparation that he has studied extensively finding it apparently identical with the agent present in the globulin fraction and active at pH 7 3–7 8 Macfarlane and Pilling (1947b) have also utilized the adsorption of the enzyme by fibrin The globulin fraction of plasma is added to a solution of fibrinogen subsequently clotted by thrombin The resulting clot is removed washed free of protein and allowed to lyse in saline when the enzyme is released into solution

Following fractionation by electrophoresis most of the enzyme is found in fraction III[2 3] (Cohn et al 1944 Edsall 1947)

ACTIVATION BY STREPTOKINASE

The procedures described above have produced activation of the plasma enzyme by destruction of its inhibitor or by separation of the enzyme inhibitor complex Recently observations have been made that provide an important link between fibrinolysis occurring as a result of these manipulations or of natural causes in the living subject and fibrinolysis produced by the culture filtrate of certain strains of β-haemolytic streptococci In 1933 Tillett and Garner had shown that human plasma if allowed to clot in the presence of such filtrates underwent very rapid fibrinolysis The plasma of patients who had recovered from streptococcal infections and rabbit plasma resisted this lysis They found however that if rabbit fibrin was clotted by human thrombin it became susceptible to lysis by the streptococcal filtrate Milstone (1941) showed that pure fibrinogen even if derived from human blood yielded fibrin which was resistant to the action of streptococcal filtrate but that if a small amount of the globulin fraction from human plasma were added lysis occurred as with whole plasma He also found that the addition of such globulin to rabbit plasma resulted in lysis of this fibrin by the filtrate He therefore postulated a lytic factor present in the globulin of human blood which was necessary to sensitize fibrin to the action of the bacterial enzyme In 1944 Kaplan came to the conclusion that Milstone s lytic factor and the proteolytic factor activated by chloroform were identical This suggestion was confirmed and extended by Christensen (1945) and Christensen and MacLeod (1945) These workers showed that the enzyme liberated in plasma or serum by the action of chloroform was the same as that which appears following the addition of streptococcal filtrate The enzyme is proteolytic being capable of digesting fibrin fibrino

gen casein and gelatin and though it resembles trypsin is distinct from it It is inhibited by the so-called trypsin inhibitor of plasma and by the pancreatic anti trypsin of Kunitz and Northrop (1936) The enzyme is present as an inert precursor associated with the globulin fraction of the plasma and its inhibitor is associated with the albumen They postulated that streptococcal filtrate contains a kinase capable of activating the precursor so that the inhibitor is overwhelmed Kaplan (1946) confirmed the distinction between the plasma enzyme and trypsin by showing that the streptococcal kinase did not activate trypsinogen nor did entero-kinase activate the plasma enzyme Macfarlane and Pilling (1946a) in observations in which plasma and plasma fractions were treated with streptococcal filtrate and chloroform came to the conclusion that plasma normally contains not only the enzyme precursor and its inhibitor but free enzyme They showed that simple dilution of whole plasma will if there is an increase in free enzyme result in the development of activity resulting from dissociation of the enzyme inhibitor complex

Terminology

In the course of previous work on the proteolytic factor of serum during the past 40 years different terms have been applied to what is almost certainly the same substance This has led to confusion and it is desirable that a unified terminology should now be adopted Such names as serum trypsin serum protease serum tryptase fibrinolysin thrombolysin and others should be abandoned in favor of a more specific designation The nomenclature proposed by Christensen and MacLeod (1945) seems to fulfil the necessary requirements In this the proteolytic enzyme of the plasma is called plasmin a choice for which good reasons are given The precursor of plasmin is termed plasminogen and the streptococcal filtrate factor previously called fibrinolysin now becomes streptokinase The antibody developed by patients recovering from streptococcal infection which is capable of neutralizing streptokinase is called antistreptokinase The inhibitor of proteolytic enzymes present in normal plasma might be called antiplasmin for the sake of uniformity though such a name implies a sense of restriction with regard to its activities which is undesirable An alternative nomenclature proposed by Loomis George and Ryder (1947) in which the term fibrinolysin is applied to the plasma enzyme seems to us to be fraught with danger of confusion since this term has in the past been applied to the streptococcal factor

The Properties of Plasmin

The identity of plasmin with the enzyme activated by chloroform apparently is established (Christensen and MacLeod 1945) The enzyme studied by Mole (1943) in cases of sudden death and that occurring in the plasma of patients after operation or other disturbance (Macfarlane and Pilling 1947b) are so similar to plasmin with regard to their physical properties and activity that it may be assumed as a working hypothesis that fibrinolysis whether induced by streptokinase chloroform fractionation or disturbances in the living subject is due to active plasmin

It has already been shown that plasmin is capable of lysing fibrin and of digest

ing fibrinogen gelatin and casein With regard to fibrinolysis though preformed fibrin may be slowly digested activity is greatly increased if the enzyme is present during the process of coagulation probably because of the strong adsorption of plasmin by fibrin during clotting Though plasmin is capable of digesting other proteins its fibrinolytic action is more rapid in proportion to such digestion than is the case with trypsin and other proteolytic enzymes Fibrinolysis therefore is a delicate indicator of plasmin activity which has been used by Macfarlane and Pilling (1946a) for quantitative estimations This method allows however of only an arbitrary expression of activity which in this case has been in terms of the greatest dilution of the enzyme that will produce lysis within 24 hours at 37 C of the clot formed by a 0 075 per cent solution of pure fibrinogen

Other methods of determination of activity used by previous workers include estimation of the increase in nonprotein nitrogen following incubation of the enzyme with a particular substrate which in some cases has been merely whole serum in others casein gelatin or hemoglobin Viscometric determinations have been made with casein and gelatin but we ourselves have found this technic unreliable even under rigidly controlled conditions Estimation of acid soluble tyrosine by the method of Anson and Mirsky (1937) has given satisfactory results We have found by this method that plasmin is capable of digesting all the proteins of normal plasma a fact which may have some significance in the physiologic effects of its activity

The pH of optimum activity of plasmin is about 7 4 and of maximum heat stability between 7 and 7 4 The enzyme is destroyed at this pH by heating to 55 C for twenty minutes It is nondiffusable through cellophane and behaves with regard to precipitation like the globulin of plasma It is precipitated by half saturation with ammonium sulphate full saturation with sodium sulphate or by bringing the pH to 5 5 after dialysing the plasma free from electrolytes It is precipitated by trichloracetic acid and by acetone and alcohol with moderate loss of activity and it is strongly adsorbed by fibrin

Inhibition of Plasmin

Antiplasmin the natural inhibitor of plasmin is associated with the albumin fraction of the plasma It is said not to be precipitated by trichloracetic acid but Duthie (1947) does not agree with this statement The inhibitor is nondiffusable through cellophane and heat labile being destroyed by heating to 66 C It is rapidly inactivated at pH values below 5 0 but is stable in the alkaline ranges It is destroyed by chloroform ether alcohol and acetone (Duthie 1947) As with pancreatic antitrypsin and trypsin the plasmin antiplasmin complex appears to be dissociable by simple dilution unless there is a considerable excess of inhibitor (Macfarlane and Pilling 1946a) and simple fractionation of the plasma into albumin and globulin is also apparently capable of splitting the complex The activity of the inhibitor must be considered in any study of fibrinolysis since the equilibrium it maintains with plasmin may be disturbed by changes in either factor We have attempted to estimate antiplasmin activity by means of the inhibitory effect of the albumin fraction to be studied on the digestion of fibrinogen and casein

by purified plasmin Plasmin is also inhibited by the crystalline antitrypsin of Kunitz and Northrop (1936) by heparin and by the soya bean trypsin inhibitor of Kunitz (1946) the latter being most effective Grob (1943) has reviewed the literature on the antiproteolytic factor of serum

Other Enzymes Possibly Related to Plasmin

The characterization of plasmin has not at present been sufficiently specific to determine whether or not certain other enzymes which have been described in body fluids and tissues are identical Since it is possible that these enzymes may enter the blood stream or like plasmin become activated from inert precursors they may be briefly considered

Hedin (1904a) identified two proteases in spleen tissue one acting at a slightly alkaline pH and inhibited by serum as was the leucoprotease of Opie and Barker (1907) There has been a large number of publications by Abderhalden (1921 1940 etc) and his associates dealing with the presence of various proteolytic enzymes in the blood and tissues which they believe to be specific for abnormal tissues Patients with carcinoma for instance produce enzymes specifically digest ing carcinomatous tissue and in pregnancy there is an enzyme capable of lysing placental tissue It is probable that part at least of these findings depend on the activation or inhibition of plasmin but the whole subject of the Abderhalden reactions is too voluminous to be dealt with adequately here Grassmann and Heyde (1930) have described peptidases in animal serum or plasma active between pH 7 and 8 that split leucylglycyl glycine which are increa ed in various pathologic states Rosenmann (1936) has extracted a fibrinolytic enzyme from liver kidney lung pancreas and thyroid tissue closely resembling his thrombolysin Masch mann (1942) has found that rats with sarcoma may have a higher level of blood peptidase than is normal Huggins Vail and Davies (1943) have studied fibrinoly sis in menstrual fluid finding considerable activity which is also present in ex tracts of endometrium prostate and thyroid tissue Zamechick Stephenson and Cope (1945) have found that lymph from the extremities of dogs contains an amino exopeptidase which is increased in amount if the limb is traumatised or burned They have also found that blister fluid from burns in human cases contains the same enzyme but Macfarlane (1943) failed to find fibrinolytic activity in blister fluid Beloff and Peters (1945) have investigated a protease extracted from human skin active at pH 6-8 This enzyme is not trypsin and does not split leucylglycyl glycine and following burning its concentration in the affected skin is decreased Beloff (1946) has studied an inhibitor of this enzyme present in the albumen frac tion of normal plasma but considers that this is distinct from antitrypsin It is not clear however that this conclusion is justified Fruton (1946) who has made an extensive study of the tissue enzymes has found two leucine aminopeptidases in lung serum and skin extracts

Macfarlane and Pilling (1947b) have examined human tissues for the presence of fibrinolytic activity The tissues were perfused if practical with running water until the vessels were blood free minced extracted with saline and the enzyme precipitated with ammonium sulphate or acid The precipitates were dissolved

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though this may develop following a variety of circumstances disturbing to the living subject The train of events connecting these stimuli with the fibrinolytic mechanism is obscure but since the nature of the latter is becoming clearer a more systematic investigation of the process has become possible since it is probable though not definitely established that fibrinolytic activity in vivo is due to plasmin

In his early experiments Nolf (1905 1908) was able to produce fibrinolysis following peptone shock in dogs in which the liver had been isolated from the circulation thus demonstrating that this organ is not a necessary link in the chain of events leading to activation Loeper *et al* (1932) found that after ligature of the renal arteries in dogs there was a loss of protein and a rise in nonprotein nitro

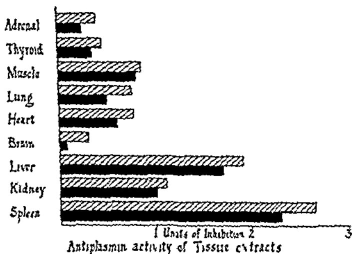


FIG 2 Histogram showing the mean antiplasmin activity of extracts of various tissues and organs (a) from cases of sudden death (solid columns)
(b) cases of slow death (hatched columns) The units refer to a dried albumin standard and do not compare to the units in Figure 1

gen in serum during incubation Hougardy (1934) has made the significant observation that removal of the pancreas does *not* reduce the proteolytic activity of the serum when treated with chloroform

Since it seemed that the condition of traumatic shock had existed in all cases in which fibrinolysis had been demonstrated and might be the prime mover of the process Macfarlane (1937) investigated the blood of patients undergoing surgical operation The technic employed used diluted citrated plasma clotted by calcium chloride it being found that in normal blood samples the fine fibrin web so formed was stable under aseptic conditions for a period of weeks In about 70 per cent of the patients after operation these fibrin webs disappeared in twenty four hours or less though control samples taken the day before from the same patients showed no undue instability The nature of the cases showing such fibrinolysis gave little information as to the factors responsible for this change There was no close correla

and tested for fibrinolytic activity. This varied from one sort of tissue to another and from case to case, but in general it was found that lung extract had the greatest activity, being in many cases relatively greater than the blood plasmin activity of the same subject. This lung enzyme resembled plasmin both as regards its physical properties and its reaction with the inhibitors, but its action on pure substrates has not been investigated. Fibrinolytic activity was also observed in extracts of kidney, suprarenal and thyroid but in a lesser degree, and it was found that in all tissues the highest yields occurred in cases of sudden death when the blood plasmin level was also increased (fig. 1).

The inhibitory power of the albumin fraction of organ extracts have also been estimated (fig. 2). It has been constantly found that spleen extract has a potent inhibitory effect on plasmin and lung extract resembling that of antiplasmin. This

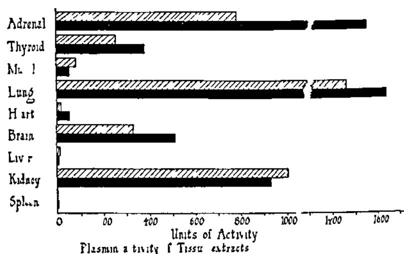


FIG. 1. Histogram showing the mean plasmin activity of extracts from various tissues and organs (a) from cases of sudden death (solid columns) (b) cases of slow death (hatched columns). The unit of activity is based on the titre of the enzyme (see text).

finding is of interest in view of the work of Ungar (1945) in which the injection of splenic extracts raised the antitryptic power of the serum.

It has been observed that there is a fibrinolytic enzyme in normal urine (Macfarlane and Pilling 1947a). This enzyme again resembles plasmin in its properties. Though the concentration of this enzyme in the urine is not closely correlated with the plasmin level of the blood, and though it differs slightly from plasmin in being heat stable and more affected by trypsin inhibitors, it is possible that it is, in fact, plasmin in the process of elimination. Other enzymes resembling pepsin have been described in urine, the literature being reviewed by Farnsworth, Speer and Alt (1946).

THE ACTIVATION OF PLASMIN IN VIVO

Though normal plasma may be activated *in vitro* by the procedures described previously, in its natural state it possesses no demonstrable proteolytic activity

activity of plasma in vitro It is clear therefore that it requires the cooperation of one or more factors which exist in the body but not in the shed blood The obvious possibility that the rise in leukocytes or platelets were directly responsible for the increase in fibrinolytic activity has been disproved by the finding that addition separately or in combination of fresh lymphocytes polymorphonuclears or platelets to normal plasma is not followed by fibrinolysis nor does the incubation of adrenalin with these elements result in any activating factor being produced *The plasma from subjects who have developed fibrinolytic activity retains* this activity after centrifuging at 20 000 rpm to remove all its formed elements Moreover in patients with marked polymorphonuclear leukocytosis lymphocytosis or thrombocytosis no spontaneous fibrinolytic activity has been observed

The injection of adrenalin in certain pathologic states has given some information if largely negative regarding the other factors concerned A normal fibrinolytic response has been obtained in two cases of Addison s disease in which subsequent postmortem examination has demonstrated almost complete destruction of the suprarenal cortex and one case of aplastic anemia in three cases of splenectomy (performed for traumatic rupture) and in three cases of hemophilia The only patients with a constantly diminished response to adrenalin are those in whom the lymph glands have been affected by Hodgkin s disease or x ray irradiation From these results and those previously mentioned therefore it appears that given the stimulus of adrenalin fibrinolysis is independent of the normal function of the suprarenal cortex spleen pancreas or liver but it is possible that lymphoid tissue may be involved

The demonstrable changes that occur in the components of the proteolytic system of plasma concurrently with spontaneous activation consist of an increase of plasmin that suggest activation of plasminogen This rise may be sufficient in extreme cases to produce fibrinolysis in the whole blood but usually it is necessary to dilute the plasma to demonstrate the increased activity A more puzzling finding is the apparent diminution in the inhibitor that also occurs in spontaneous activation Figure 5 illustrates the reduction of the inhibitory effect of the albumen fraction of normal plasma as compared with plasma from the same subject following activation by adrenalin It appears therefore that the process of activation in vivo is more complex than the mere secretion into the blood stream of a kinase capable of activating plasminogen Such a kinase however is probably concerned and it is of interest that Astrup and Permin (1947) claim to have observed its presence in fresh tissues though it is not clear that they have eliminated t e active enzyme already present in such preparations

The Significance of Fibrinolysis

The balance between proteolysis and its inhibition may vital processes in which blood constituents are involved The are the proteolysis of pathologic fibrin deposits and the dest teins following the activation of plasmin In the more com blood coagulation both proteolytic and inhibitory factors clotting and some naturally occurring anomalies may be rela

tion between fibrinolysis and the traumatic shock experienced by the patient since quite minor operations might be accompanied by relatively intense activity whereas major surgical procedures might give negative results Similar results were obtained by the same technic by Imperati (1937) though the rather lower incidence of 50 per cent of fibrinolysis following operation was recorded He too observed the lack of correlation between the severity of the operation and the degree of fibrinolytic activity From these investigations it appeared that fibrinolysis is dependent on some factor commonly associated with the procedure of surgical operation though not obviously connected with shock or trauma but the design of the original experiment was not sufficiently controlled to allow of its identification by analysis of its results

A further experiment was therefore carried out (Macfarlane and Biggs 1946) in which serial blood samples were obtained from each patient to control the effect of premedication and the anaesthetic as well as the operation itself It was found that a high proportion of positive results were obtained before the operation and even before the anesthetic or premedication which could not be explained by the pathologic condition from which the patients were suffering since trivial conditions involving no constitutional disturbance were included The one common factor seemed to be preoperation anxiety a conclusion which was borne out by the independent observations of Lattner (1947) who had found fibrinolytic activity in cases of anxiety states and hyperthyroidism in a normal person during an air raid Kaulla (1947) found that fibrinolysis occurred after the injection of novocain or even a large volume of saline and though he believed that displacement of tissue fluid was the activating agent in this latter experiment the effect of anxiety cannot be excluded In addition to the operation cases it was found that positive fibrinolysis occurred in a variety of pathologic states in which hypersensitivity appeared to be the common factor but in view of the probable effect of anxiety such findings are not easy to assess

The next observations (Biggs Macfarlane and Pilling 1947) concerned the discovery that severe exercise induced fibrinolytic activity in normal subjects This was proportional to the amount of exercise taken and to the degree of exhaustion it induced the latter being influenced by training The occurrence of fibrinolysis both in exercise and in anxiety suggested of course that adrenalin activity might be an important underlying factor and experiment established that intense fibrinolytic activity could be induced in normal subjects by the injection of adrenalin The findings resembled closely those provided by exercise since in both fibrinolytic activity appeared in the circulating blood within a minute or so of the onset of the stimulus and disappeared within a few minutes of its cessation and in both there was a change in the blood picture consisting of an almost immediate rise in lymphocytes with a slower rise in polymorphonuclear leukocytes and platelets These results provided an explanation of previous observations since in all cases in which fibrinolytic activity has been recorded it is likely that adrenal activity will have been stimulated It must be determined of course what part adrenalin plays in the reaction We have made observations on this matter b they are far from complete at present Adrenalin itself produce

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The Significance of Fibrinolysis

The balance between proteolysis and its inhibition may control many of the vital processes in which blood constituents are involved The most obvious of these are the proteolysis of pathologic fibrin deposits and the destruction of plasma proteins following the activation of plasmin In the more complicated mechanism of blood coagulation both proteolytic and inhibitory factors can alter the speed of clotting and some naturally occurring anomalies may be related to changes in the

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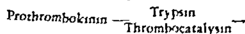
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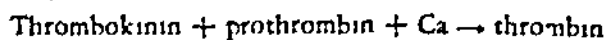
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which might produce plasmin activity from destruction of antiplasmin there is a demonstrable fall in fibrinogen in five hours (Whipple 1914) and a profound deficiency in three days (Whipple and Hurwitz 1911 Whipple 1914 Smith Warner and Brinkhouse 1937) This disappearance of fibrinogen though usually attributed entirely to failure of regeneration is more rapid than that which occurs without a toxic factor as for instance when the normal liver is excluded from the circulation (Whipple 1914) or following transfusion in a patient with afibrinogenemia when fibrinogen could still be d monstrated in the circulation after eight days (Pinniger and Prunty 1946) Denys and Marbaix (1889) were the first to suggest that the loss of nitrogen which occurs in chloroform poisoning might be due to proteolysis Opie Barker and Dochez (1911) showed that there was an increase in nonprotein nitrogen in the incubated serum from an animal with chloroform poisoning and Jacoby (1900) demonstrated fibrinolysis in severe phosphorus poisoning It is ther fore probable that proteolysis is a factor in the loss of fibrinogen in chloroform and phosphorus poisoning

Blood Coagulation

There is a considerable accumulation of evidence in favor of the view that one or more components of the fibrinolytic system are concerned in blood coagulation The fact that chloroform serum causes the clotting of fibrinogen solutions originally suggested to Nolf (1908 1938 etc) that proteolysis might be a factor in normal blood coagulation He considered that two substances thrombogen and thrombozyme combined in varying proportions with fibrinogen to form a clot an excess of thrombogen and thrombozyme leading to the release of a proteolytic enzyme which might cause incoagulability from destruction of fibrinogen or coagulation followed by fibrinolysis The coagulant action of the globulin fraction observed by Tagnon (194-) suggests the participation of plasmin in blood clotting and although its exact role has not been determined the facts available suggest an analogy between the action of plasmin and trypsin Neither purified plasmin nor trypsin will clot fibrinogen solutions (Dale and Walpole 1916 Seegers and Loomis 1947) and both apparently lead to the relea e of thrombin from oxalated plasma (Dale and Walpole 1916 Stephen and Wohl 19-1) A further study of trypsin in relation to coagulation is therefore of interest Ferguson and Erickson (1939) have shown that trypsin causes an increased rate of conversion of prothrombin to thrombin and Lenggenhager (1946) showed that pure prothrombin is converted into thrombin by trypsin only in the presence of an additional plasma component which he has called prothrombokinin The amount of thrombin formed by trypsin is directly proportional to the amount of prothrombokinin present He suggests that trypsin activates prothrombokinin to a substance called thrombokinin which combines directly with prothrombin in the presence of calcium to form thrombin Lenggenhager extracted a natural activator of prothrombokinin (thrombocatalysin) from plasma and found that this was fibrinolytic According to Lenggenhager thrombin formation proceeds as follows





plasmin antiplasmin complex A normal balance of proteolytic and inhibitory substances in the blood may also be necessary for the dynamic equilibrium between blood and tissue proteins which probably forms the background of protein metabolism and the negative nitrogen balance which follows alarming stimuli such as trauma burns etc may be related to the plasmin activity with which they are frequently accompanied Proteolysis may also under certain circumstances lead to the liberation of harmful products of protein digestion and the release of histamine in anaphylactic shock has been attributed to this cause

The Dissolution of Fibrin

In local inflammation or trauma fibrin is formed in the tissues and during healing its necessary reabsorption is probably due either to the action of plasmin or

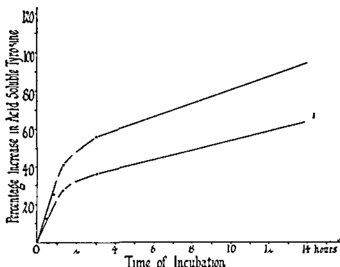


Fig 3 Curves showing the digestion of casein by plasmin in terms of the release of acid soluble substances estimated as tyrosine In each case a similar concentration of the albumin fraction from the blood of the same subject (1) resting and (2) after adrenalin injection has been added A reduction in anti plasmin effect is observed following the administration of adrenalin

to the proteolytic enzymes of the tissues Similarly in pneumonia relatively large amounts of fibrin are liquified during resolution and it is significant that the lung often contains an unusually high content of fibrinolytic enzyme Following intra vascular thrombosis the thrombus may disintegrate a process that may be accompanied by general fibrinolytic activity which Kaulla (1947) has demonstrated in the blood of patients with thrombosis during clinical improvement The dissolution of clots by fibrinolytic enzymes in the endometrium may be a factor in normal menstruation (Smith and Smith 1945)

Chloroform Poisoning

The destruction of fibrinogen by plasmin can seldom be demonstrated in vivo because the normal liver replaces fibrinog n with great rapidity so that when the blood of dogs is replaced by defibrinated blood normal coagulation is restored within an hour (Whipple 1914 Goodpasture 1914) In chloroform poisoning

Inhibition by the albumin fraction in hemophilia was studied by Feissly (1944) He showed that whereas the coagulation time of whole blood in hemophilia is greatly prolonged that of the hemophilic globulin fraction is only slightly longer than normal He then showed that hemophilic albumin retards the clotting of both normal and hemophilic globulin fractions These experiments which suggest an increase in the inhibitory system in hemophilia are supported by the work of Tocantins (1943) who postulates an increase in antithromboplastin

A direct relationship between a shortening of the coagulation time and fibrinolysis occurs following adrenalin injections Cannon and Mendenhall (1914 a & b) showed that adrenalin stimulation of the sympathetic nervous system and emotional stimuli lead to a shortening of the coagulation time of recalcified plasma This hastening of coagulation is apparently not due to any change in prothrombin because Wakin et al (1946) have shown that adrenalin causes no alteration in the Quick prothrombin time It therefore seems possible that the change in coagulation time may be related to an increase in thromboplastin Since there is now considerable evidence that thromboplastic action is in part proteolytic and since adrenalin injections consistently produce fibrinolysis it seems possible that this shortening of the coagulation time may be related to fibrinolysis

The Alarm Reaction of Selye

The suggestion that fibrinolysis occurs in all conditions which lead to secretion of adrenalin has interesting implications in relation to Selye s concept of the alarm reaction (Selye 1946 etc) According to Selye exposure to any noxious or excessive stimulus is followed by a uniform pattern of pathologic changes There is an initial shock phase with the characteristics usually associated with this term and a succeeding counter shock phase when the phenomena of shock are reversed The reaction is accompanied by involution of the lymphoid tissue hypertrophy of the adrenal cortex and excessive secretion of adrenal cortical hormones

The marked negative nitrogen balance of the alarm reaction which may be related to plasmin has been studied in detail following trauma (Cuthbertson 1930 1932 1935 Vaughan et al 1947) burns (Cope et al 1943 Taylor et al 1943 Chanutin and Gjessing 1946) operations (Chanutin et al 1938) hemorrhage (Elman 1944) and infections (Coleman and DuBois 1915) There is an extensive loss of nitrogen from the tissues an immediate and progressive fall in plasma albumin and from the second or third day a rise in plasma globulin which often continues until the albumin globulin ratio is reversed The early fall in plasma albumin might be due to fibrinolysis initiated by adrenalin The continuing destruction of protein reserves might be related to the excessive secretion of adrenal cortical hormones which are known to cause mobilization of globulins from the tissues (White and Dougherty 1945) The reversed albumin globulin ratio thus produced might be associated with an imbalance of the plasmin antiplasmin complex causing further catabolism of protein

Fibrinolysis and Anaphylaxis

Rochas e Silva et al (1946 a b & c) have suggested that fibrinolysis may play an important part in anaphylaxis It is well known that intravenous injections of

Lenggenhager's views are supported by various observations Feissly (1942) obtained a proteolytic component from the acido globuline fraction of Doladilhe (1937) which had the power to convert prothrombin to thrombin in the presence of calcium He concluded that this substance was one of two components of thromboplastin the second being a phospho lipoid Fantl and Nance (1946) and Owren (1947) both showed that an additional factor from the plasma was necessary for the conversion of prothrombin to thrombin

The fact that plasmin and trypsin can coagulate oxalated plasma in the absence of added calcium makes it difficult to accept coagulation by these substances as closely analogous to that occurring normally However Ferguson and Erickson (1939) have shown that optimum coagulation with trypsin requires the addition of both calcium and cephalin and Lenggenhager (1946) showed that trypsin even in concentrations of 0 25–1 per cent will not coagulate plasma which is continually in motion and suggested that its coagulant action on citrated plasma was due to a local accumulation of calcum ions released by proteolysis from an inactive form in combination with protein Moreover although chloroform serum will coagulate plasma and fibrinogen there is some doubt as to whether purified plasmin has this power (Macfarlane and Pilling 1947b) Thus calcium may be necessary for coagulation with both trypsin and plasmin The difficulty raised by Rosenmann (1937) who showed that substances probably identical with plasmin and antiplasmin could be distinguished from all known coagulant factors does not preclude the possibility that plasmin may activate an additional plasma factor not recognised by him The present evidence therefore supports the view that plasmin may be concerned in the activation of prothrombin a suggestion which implies that one of the components of thromboplastin is proteolytic

Indirect evidence for the proteolytic theory of thromboplastin was suggested by observations on the coagulation defect in hemophilia Tagnon Davidson and Taylor (1942) showed that the hemophilic globulin could not be activated by chloroform Feissly (1942) showed that the globulin fraction was not proteolytic and Ferguson (1939) showed that hemophilic prothrombin could be converted into thrombin in the normal time on the addition of trypsin These observations appear to suggest a deficiency in the proteolytic component of thromboplastin but the experiments of Tagnon and Feissly could not be confirmed by Macfarlane and Pilling (1947b) and Tagnon's results have been refuted by Lewis et al (1946) Furthermore if hemophilic patients receive an injection of adrenalin they develop a normal fibrinolytic reaction with only a slight reduction in the coagulation time of whole blood and recalcified plasma (Biggs Macfarlane and Pilling 1947)

A study of the inhibitors of proteolysis also supports the view that proteolysis is a factor in coagulation Ferguson (1942) and Grob (1943) showed that pancreatic trypsin inhibitor delays coagulation and Grob (1943) showed that the delay results from an inhibition of either prothrombin or thromboplastin Macfarlane and Pilling (1946b) and Macfarlane (1947) studied soya bean trypsin inhibitor and showed that its inhibitory effect on blood coagulation was antithromboplastic By inference these observations suggest that thromboplastin has a proteolytic component

5 Enzymes resembling plasmin have been found in various tissues particularly lung and in urine

6 Inhibitors resembling antiplasmin have also been found in various tissues particularly the spleen

7 Plasmin activity may occur in the living subject following various stimuli such as trauma fear severe exercise or the injection of adrenalin

8 *The mechanism of this activation is still obscure and involves factors not* present in whole blood It can be obtained in the absence of the normal function of the liver pancreas spleen and suprarenal cortex

9 Such plasmin activity may be concerned in the phenomena of shock in the equilibrium between protein breakdown and synthesis in the mechanism of blood coagulation and in allergic reactions

10 The position may be illustrated diagramatically as shown in figure 4

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trypsin cause profound shock (Tagnon 1945) Rochas e Silva and Grana (1946a) showed that trypsin perfused through the isolated liver causes the release of histamine On the other hand an anaphylactic antigen caused no release of histamine from the livers of sensitized animals unless it was perfused in whole blood Since anaphylactic shock is associated both with a rise in blood histamine and fibrinolysis (e Silva and *Teixeira 1946b) they suggested that in contact with sensitized* tissue the anaphylactic antigen releases fibrinolysin which in turn releases histamine from the liver cells In 1947 Ungar showed that incubation of sensitized tissue with the specific antigen does cause a release of proteolytic enzyme and in 1945 he showed that resistance to trauma was associated with a decreased release of histamine from the cells and an increase in blood antitrypsin These observations clearly support the original suggestion of Rochas e Silva

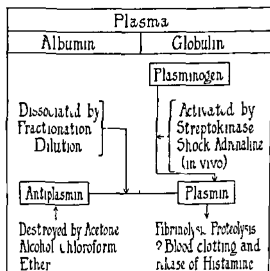


FIG 4 Diagrammatic representation of the activation of plasmin and its effects

There is therefore cumulative evidence that the plasmin antiplasmin complex plays a fundamental part in several essential physiologic processes

CONCLUSIONS

1 There exists in normal blood a proteolytic enzyme plasmin its precursor plasminogen and its inhibitor antiplasmin the latter being in excess

2 Plasmin and plasminogen are associated with the globulin fraction of the plasma antiplasmin with the albumin

3 Plasmin activity can be produced in plasma in vitro by the action of streptokinase which activates plasminogen by chloroform which destroys antiplasmin or by procedures such as fractionation of the plasma which separates the plasmin antiplasmin complex

4 Active plasmin will cause fibrinolysis digestion of the plasma proteins and casein and gelatin

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The occurrence in the serum of a factor which participated in the coagulase reaction was also observed in this laboratory confirming the report of Smith and Hale[7] This factor has been extracted from human plasma and its nature and properties examined in an attempt to elucidate further its role in the clotting mechanism In addition it has also been observed that an inhibitor for coagulase could be obtained from normal human plasma and from the plasma of certain species of animals This inhibitor appeared to play a significant role in determining the susceptibility of these plasmas to the action of coagulase In the present report the properties and action of the activator and inhibitor for coagulase are described

Materials and Methods

1 *Staphylocoagulase* The source of coagulase throughout this study were viable cultures of coagulase positive strains of staphylococci which had been grown in tryptose phosphate broth for 48 hours

2 *Fibrinogen* * The fibrinogen preparation employed was a dried product which had been precipitated repeatedly from pooled normal human plasma by the method of alcoholic fractionation[8] A 2 per cent solution of the dried powder was used

3 *Thrombin* The preparation was a 1 10 dilution of a commercial product derived from rabbit blood †

4 *Plasma defibrinated by heating* Oxalated or citrated plasma was heated to 56 C kept at this temperature for three minutes and then immediately cooled in running water The flocculated fibrinogen was removed by centrifuging The defibrinated plasma thus obtained possessed no thrombic activity for fibrinogen

5 *Plasma coagulase test* Two-tenths ml of a forty eight hour culture of a given strain of staphylococcus was added to 0 5 ml of citrated or oxalated plasma and the tube placed in a water bath at temperature 37 C Periodic observations were made to note the time and degree of clotting A test was considered positive only if a cohesive clot was formed within twenty four hours while a test was considered negative if no clot was formed after incubation for twenty four hours

Experimental

I PARTICIPATION OF A PLASMA FACTOR IN THE STAPHYLOCOAGULASE REACTION

Fresh human plasma has been generally employed as a substrate for use in the routine staphylocoagulase test Because of the availability however of such products as frozen plasma dried plasma and dried fibrinogen it was of interest to determine whether these materials could be substituted for fresh plasma in the test Accordingly a series of pathogenic strains of staphylococci were tested with these substrates as well as with fresh human plasma Using fifteen different strains as a source of coagulase it was found that the substitute substrates were much less susceptible to coagulation than the fresh plasma As shown in table 1 of the fifteen *strains which were coagulase positive when tested with fresh human* plasma only seven clotted plasma which had been rethawed from the frozen state three clotted reconstituted dried plasma and none clotted solutions of lyophilized fibrinogen even after incubating for a period of twenty four hours Moreover in those instances in which clotting of the frozen or dried samples of plasma occurred the clotting time was prolonged ranging from twelve to twenty four hours as contrasted with one to two hours for samples of fresh plasma The relative refractoriness of these three substrates to clotting by coagulase could not be attributed to a deficiency of clottable fibrinogen since all three substrates were readily clotted by such agents as thrombin snake venom and papain within two to eight minutes

* The fibrinogen preparation was kindly supplied by Sharp and Dohme Inc

† *Hemostatic Globulin* Lederle Laboratories Inc

STUDIES OF THE STAPHYLOCOAGULASE REACTION NATURE AND PROPERTIES OF A PLASMA ACTIVATOR AND INHIBITOR

By MELVIN H KAPLAN, B S AND WESLEY W SPINK M D

SINCE Loeb s[1] observation in 1903 that certain strains of staphylococci possessed the property of clotting oxalated plasma, considerable study has been devoted to the mechanism of the staphylocoagulase reaction and to its possible role in staphylococcal infections The ability to clot oxalated plasma has been found to be a property generally associated with pathogenic strains of staphylococci however the relationship between clotting activity and pathogenicity has not been clearly understood Studies of the mechanism of the clotting action of staphylocoagulase have yielded several conflicting hypotheses

Gengou[2] and Vanbreuseghem[3] noted that the clots produced from plasma by staphylococci underwent dissolution Purified fibrinogen preparations were not clotted but underwent lysis without clot formation In view of these observations both authors agreed that the coagulant activity of staphylococci could be attributed to a fibrinolysin whose action resulted in clotting only in the presence of a suitable plasma inhibitor Gratia[4] proposed the term coagulase for the clotting agent elaborated by staphylococci and reported that the participation of prosero zyme (prothrombin) or cytozyme (thromboplastin) was not necessary for the action of coagulase plasma freed of these substances by adsorption methods remained clottable He also noted that purified fibrinogen could not be clotted by staphylococci but clotting occurred readily when a small amount of a plasma preparation was added which had been freed of its content of fibrinogen prosero zyme and cytozyme Gratia concluded that the clotting activity of staphylococci was due to the elaboration of coagulase but for the elaboration of this agent a nutrient factor was required which was not present in purified fibrinogen preparations but was present in plasma However in opposition to these reports Cruickshank[5] and Walston[6] both reported that coagulase acted on fibrinogen directly and was therefore similar in its action to thrombin In the most recent studies Smith and Hale[7] confirmed the earlier reports and showed that fibrinogen was not clotted by cell free coagulase unless there were added small amounts of human or rabbit plasma serum or testicular extract The accessory clotting factor present in the plasma and testicular extract was not identical with either prothrombin or thrombokinase The authors proposed that the function of this factor was to convert staphylocoagulase into an active thrombin like substance and they therefore termed it activator It was also suggested that the relative insusceptibility of the plasmas of certain animal species to the clotting action of coagulase could be attributed to a deficiency of activator in these plasmas

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Aided by a grant from Sharp and Dohme Inc
Dr Robert Pennell and Dr W F Verwey of the Medical Research Division Sharp and Dohme prepared and supplied several preparations used in these studies

have destroyed the fibrinogen at the end of twenty four hours as was indicated by the fact that the addition of thrombin did not result in a clot The other three strains apparently exerted no appreciable fibrinolytic effect in the twenty four hour period since fibrin clots were obtained following the addition of thrombin Similar results have been obtained with a larger series of tests and it was therefore concluded that the failure of preparations of fibrinogen to be clotted by coagulase was not due to the action of a fibrinolysin but rather to the deficiency of an accessory clotting factor

This clotting factor as it has been previously pointed out has been termed an activator of coagulase by Smith and Hale [7] In the present state of knowledge

TABLE 2 —*The effect of addition of defibrinated plasma on the clotting of fibrinogen by coagulase*

Strain of Staph. aureus	Substrate Mixture				Clotting time
	Plasma	Fibrinogen	Defibrinated Plasma	Saline	
	ml	*ml*	*ml*	*ml*	*h*
Sch	0 5			0 3	1
Sch		0 5		0 3	>24 (c)†
Sch		0 5	0 3		–
Rut	0 5			0 3	1
Rut		0 5		0 3	>24 (c)
Rut		0 5	0 3		2
Bur	0 5			0 3	1
Bur		0 5		0 3	>24 (nc)
Bur		0 5	0 3		–
Ter	0 5			0 3	1
Ter		0 5		0 3	>24 (c)
Ter		0 5	0 3		2
Lou	0 5			0 3	1
Lou		0 5		0 3	>24 (nc)
Lou		0 5	0 3		2

The defibrinated plasma was prepared by heating normal human plasma at 56 C for 3 minutes

† (c) represents clot formation on addition of thrombin and (nc) represents no clot formed on addition of thrombin

such a *function should be regarded as highly hypothetical however for purposes* of uniformity the term activator has been retained in the present report

2 TESTS OF PLASMA FRACTIONS FOR ACTIVATOR CONTENT

In order to determine which of the plasma protein fractions contained the activator human citrated plasma was fractionated into five components at increasing saturation with ammonium sulfate and each fraction then tested for its content *of activator* Precipitates were obtained at *25 40 60 80 and 100 per cent* saturation with ammonium sulfate Each precipitate was washed once in the appropriate concentration of ammonium sulfate and redissolved in distilled water to a volume one half that of the original plasma The solutions were then dialyzed against physiological saline solution in the cold for eighteen to twenty four hours

The method of titration for activator was as follows Serial dilutions with physiological saline solution were made with each fraction in 0 5 ml volumes To each dilution was added 0 5 ml of a forty

A possible explanation for the apparent resistance of these samples of fibrinogen and plasma to clotting was that they were deficient in some clotting factor present in fresh plasma. Prothrombin was excluded as this factor since the Quick[9] prothrombin levels of both the frozen and dried plasma were practically the same as that of fresh plasma, i.e., 12.8, 12.3 and 12.5 seconds respectively. Tests for an accessory clotting factor were therefore carried out with fresh human serum. As a test substrate 0.2 ml. of fresh human serum was mixed with 0.5 ml. of fibrinogen and susceptibility to clotting determined by addition of 0.2 ml. of a staphylococcus culture. It was at once apparent that the addition of serum rendered the fibrinogen readily clottable by coagulase. Further, the clotting which resulted was not due to the presence of thrombin in the serum, since interference by thrombin could be excluded by suitable dilution of the serum or by destroying its thrombic activity

TABLE 1.—*Comparative susceptibility of fresh plasma, frozen plasma, dried plasma, and dried fibrinogen to clotting by 15 strains of pathogenic staphylococci*

No.	Strain of Staphylococcus	Coagulase Test			
		Fresh Plasma	Frozen Plasma*	Dried Plasma	Dried Fibrinogen
1	Wro	+	+	+	−
2	May	+	−	−	−
3	St A	+	+	−	−
4	Lin	+	−	−	−
5	Ver	+	+	−	−
6	Sha	+	−	−	−
7	Sch	+	+	+	−
8	Lon	+	+	+	−
9	Slo	+	−	−	−
10	Ter	+	−	−	−
11	Cos	+	−	−	−
12	Ede	+	−	−	−
13	Pew	+	+	−	−
14	Rut	+	−	−	−
15	Nel	+	+	−	−

* This plasma was stored in the frozen state for two years before thawing.

by heating at 60 C. for thirty minutes. These procedures still permitted the activity of the accessory coagulase factor to be readily demonstrated.

The most satisfactory source of this accessory factor was plasma from which the fibrinogen was removed either by heating to 56 C. for three minutes or by precipitation at one fourth saturation with ammonium sulfate. These preparations had the advantage that they possessed no thrombic activity. As shown in table 2, when 0.2 ml. of such a defibrinated sample of plasma was added to fibrinogen, clotting occurred almost as readily as with the fresh plasma. Fibrinogen to which no defibrinated plasma had been added was not clotted by any of these strains. This failure to clot could not be attributed simply to the destruction of the fibrinogen by the fibrinolytic action of the staphylococci, as had been suggested by Gengou. Indeed, of the five strains included in table 2, only two were found to

ammonium sulfate To 1 volume of fresh citrated human plasma was added slowly with continuous stirring 1 5 volumes of saturated ammonium sulfate and the mixture allowed to stand for four hours at temperature 4 C This precipitate obtained at 60 per cent saturation was removed by filtration in the cold and discarded To the supernate solid ammonium sulfate was added to saturation and the mixture was usually allowed to stand overnight in the refrigerator before filtering The fraction thus obtained between 60 and 100 per cent saturation was dissolved in a small amount of distilled water and dialyzed against physiological saline solution for eighteen hours in the cold The resulting solution was then dried from the frozen state by the lyophile process and the resulting light yellow powder used

TABLE 4 — *Activator titer of human plasma fractions obtained by alcoholic fractionation*

No	Alcoholic Fraction	Titer of Activator*
1	I	0
2	II + III	0
3	IV 1	0
4	IV 4	8
5	V	2
6	Crystalline albumin	0

* Highest dilution which when mixed with fibrinogen solution permitted clotting by coagulase in four hours

TABLE 5 — *Thermolability of Activator*

No	Temperature to which preparation was exposed for 30 min	Titer of Activator	
		Defibrinated Plasma	Activator Preparation (Am Sulfate Fraction)
1	Control (not heated)	8	8
2	55 C	8	8
3	65 C	8	4
4	75 C	8	2
5	100 C	4	2
6	Boiling	0	0

without further purification It was found to be completely stable for at least a month when stored in the cold Solutions of activator employed in the present study were usually made up in a concentration of 200 mg of dried preparation per 10 ml of distilled water

4 PROPERTIES OF ACTIVATOR

A *Thermolability* Smith and Hale[7] found the activator present in rabbit testicular extracts to be completely inactivated after heating at 56 C for thirty minutes Such thermolability however has not been observed with activator derived from human plasma Heat sensitivity tests using both defibrinated plasma and am

eight hour broth culture of a standard strain of staphylococcus (Bur) and incubation carried out for one hour in a water bath at 37 C To each tube was added 0 5 ml of fibrinogen solution the tubes shaken and incubation carried out for four hours The titer of activator in the sample was taken as the highest dilution which yielded a solid cohesive matty clot

As shown in table 3 the activator was concentrated essentially in the fraction obtained between 80 and 100 per cent saturation while the fraction between 60 and 80 per cent saturation possessed smaller amounts of activity Activator thus appeared to be associated with the crude albumin fraction of the plasma

A more precise characterization of the nature of activator was made possible by testing human plasma fractions prepared by alcoholic fractionation * It has been shown by Cohn and collaborators[8] that the plasma proteins may be separated into five fractions on the basis of their differential solubility in ethanol water mixtures at low temperatures and at varying pH and ionic strength These five fractions I II+III IV 1 IV 4 and V as well as a sample of crystalline serum albumin were

TABLE 3 —*Activator titer of human plasma fractions prepared by ammonium sulfate precipitation*

No	Plasma Fractio	Titer of Act ato
1	25% sat $(NH_4)_2SO_4$	0
2	25–40% $(NH_4)_2SO_4$	0
3	40–60% $(NH_4)_2SO_4$	0
4	60–80% $(NH_4)_2SO_4$	4
5	80–100% $(NH_4)_2SO_4$	16

Highest dilution of the test substance which when mixed with fibrinogen permitted clotting by coagulase in four hours

titrated for their relative content of activator The solutions in each case were made up to contain 200 mg of the dried fraction in 10 0 ml of $\frac{1}{20}$ M phosphate buffered saline pH 7 4

As shown in table 4 activator was found to a significant extent only in fractions IV 4 and V The greater amount of activity appeared in fraction IV 4 which is made up chiefly of alpha and beta globulins Fraction V which is essentially albumin contained a somewhat smaller amount of activator while crystalline albumin was without activity

From the above findings it appeared that activator is probably not an albumin although it is precipitated with this protein fraction by ammonium sulfate In plasma fractions obtained by alcoholic fractionation activator appeared to be associated with the alpha and beta globulins

3 PREPARATION OF DRIED ACTIVATOR MATERIAL FROM CITRATED HUMAN PLASMA

The activator preparations used in the subsequent studies were prepared from the fraction of plasma precipitated between 60 and 100 per cent saturation with

* These fractions were supplied by Dr E J Cohn and his associates The materials were made possible through contracts between Harvard University and the Office of Scientific Research and Development and the plasma was obtained through the American Red Cross and more recently through the Massachusetts Civilian Blood Donor Program

5 PRESENCE IN NORMAL HUMAN PLASMA OF A SUBSTANCE INHIBITING THE COAGULASE REACTION

It was noted that when some samples of human defibrinated plasma were employed as a source of activator clot formation occurred much more rapidly when the samples were diluted This same accelerating effect was observed also on diluting some activator preparations One given activator preparation for example when mixed with fibrinogen and coagulase permitted clotting to occur in eight hours but when this same preparation was diluted 16-fold clotting occurred in two hours This observation suggested the presence in these activator preparations of an inhibitor which interfered with clot formation and that the action of the inhibitor could be suppressed by suitable dilution * It was noted however that the inhibitory effect did not occur with all preparations of activator

On fractionation of human oxalated plasma by means of ammonium sulfate the inhibitor was found to be present in the globulin fraction obtained at half saturation The inhibitory action of such a globulin preparation is demonstrated in the following experiment One tenth ml of a 4 times concentrated human globulin preparation was added to a mixture containing 0.5 ml of activator solution 0.5 ml of coagulase and 0.5 ml of fibrinogen and the clotting time noted The result

Table 7 — Inhibitory effect of a plasma globulin factor on the coagulase reaction

Globulin	Activator	Coagulase	Saline	Clotting Time
ml	*ml*	*ml*	*ml*	*hr*
0.1	0.5	0.5	0	>24
0	0.5	0.5	0.1	2

was compared with a control test in which 0.1 ml of saline solution was substituted for the 0.1 ml of test preparation As shown in table 7 the mixture containing the plasma globulin preparation was not clotted even after twenty four hours while a clot appeared in the control tube in two hours The coagulase reaction was thus entirely inhibited by the globulin preparation

In order to compare quantitatively the content of inhibitor in different plasma fractions a method was devised for determining inhibitor titer The inhibitory titer of a given sample was measured as follows Serial dilutions of the test sample were made in saline solution in 0.5 ml volumes To each tube 0.5 ml of activator solution and 0.3 ml of coagulase was added and these mixtures were then incubated for sixty minutes Finally 0.5 ml of fibrinogen was added and the tube incubated for four hours The highest dilution of the test sample preventing clotting completely at the end of the four hour period was taken as the inhibitory titer

Table 8 demonstrates that practically all of the inhibitor present in human plasma is associated with the fraction obtained at 25 to 40 per cent saturation with ammonium sulfate When such globulin preparations were dialyzed against dis

* The greater susceptibility to clotting of plasma which is diluted also led Lominski and Roberts to the recognition of an inhibitor in the plasma which they have independently described [10]

monium sulfate fractions as a source of this material have indicated no apparent inactivation following exposure to 56 C for thirty minutes Indeed, as shown in table 5 complete inactivation of defibrinated plasma did not occur even after heating at 100 C for thirty minutes although there was some loss of activity Strong boiling for thirty minutes caused complete destruction

B *Stability at various pH* Solutions of activator were markedly stable within a wide range of acid and alkaline pH The effect of pH variation was determined by mixing given amounts of activator with a series of buffers from pH 2 0 to 10 0 and then permitting these mixtures to stand for four hours at room temperature before neutralizing The mixtures thus treated showed no measurable loss of activator titer

C *Inactivation by pepsin and nitrous acid* Since activator was stable at acid pH it was possible to determine whether it could be destroyed by the proteolytic action of pepsin Accordingly 1 0 ml *of activator solution was adjusted to* pH 3 0 and incubated with 1 0 ml of a 0 05 per cent solution of pepsin (Merck) for

TABLE 6 —*The inactivation of activator by treatment with (1) pepsin and (2) nitrous acid*

Expt No	Reaction Mixture	Activator Titer
1	(a) Activator + pepsin	0
	(b) Activator + saline control	8
2	(a) Activator + nitrous acid	0
	(b) Activator + saline control	4

thirty minutes at 37 C At the end of this period pepsin action was stopped by neutralizing the mixture with $\frac{1}{5}$ M phosphate buffer pH 7 5 *The resulting mixture* was then titrated for its activator content As shown in table 6 activity of activator was completely lost by this treatment while control samples in which saline solution was substituted for pepsin and which were subjected to the same temperature and pH remained fully active

Treatment of activator solutions with nitrous acid also resulted in rapid inactivation On addition of dilute sodium nitrite solution to activator preparation at pH 3 0 complete inactivation was observed within fifteen minutes (table 6) Since the action of nitrous acid within this period is primarily directed against alpha *amino groups it would appear that the action of activator is dependent* upon the integrity of alpha amino groups in the molecule

The data obtained would indicate that activator is a relatively stable protein constituent of the plasma It is precipitated between 60 and 100 per cent saturation with sulfate but in alcoholic fractions it is associated more closely with the alpha and beta globulins It is nondialyzable is not readily destroyed by heating below 100 C and is entirely stable between pH 2 0 to 10 0 Its activity is completely destroyed by nitrous acid or by the proteolytic action of pepsin

As shown in table 9 the rabbit preparation contained no inhibitor by the method employed while both the human and guinea pig globulin preparations possessed a titer of 8 The absence of inhibitor in the rabbit was interesting in view of the marked susceptibility of the plasma of this species however an inverse relation ship between inhibitor level and susceptibility did not seem to be general as was indicated by the similar levels of inhibitor in human and guinea pig plasmas

Since susceptibility must depend also on the presence of activator in the plasmas the plasma of each of these three species was next analyzed for their content of activator Titrations were performed both on defibrinated samples and on the albumin fractions separated between 60 and 100 per cent saturation Each albumin precipitate was dissolved in distilled water and brought up to the original plasma volume so that the concentration of activator was presumably the same as that in the plasma

As shown in table 9 the activator titer of the guinea pig plasma was significantly lower than either the human or rabbit plasma Thus the relative titers of activator and inhibitor in the plasmas of these species appeared to explain their degree of susceptibility to the action of coagulase Rabbit plasma which was most susceptible

TABLE 9—*The levels of inhibitor and activator in human rabbit and guinea pig plasmas*

Plasma of Species Tested	Inhibitor Titer of Globulin Fraction	Activator Titer of Defibrinated Plasma	Activator Titer of Albumin Fraction
Human	8	8	8
Rabbit	0	16	8
Guinea Pig	8	4	2

contained a high level of activator and no inhibitor while guinea pig plasma which was less susceptible than that of the rabbit contained less activator and a more elevated level of inhibitor The susceptibility of the human plasma which also contained an elevated level of inhibitor may perhaps be explained by the high level of activator which it possessed

The action of the inhibitor did not seem to be specifically exerted on activator This conclusion followed from the repeated observations that plasma samples could be readily separated into inhibitor-containing and activator containing fractions the activities of which were entirely independent of each other Further as shown in table 9 the separation of activator from defibrinated plasma did not result in an increase in the titer of activator as might be expected if activator were freed from an inhibitor The evidence therefore suggests that the inhibitor does not combine with activator but acts on some other factor in the coagulase reaction presumably coagulase

DISCUSSION

The present report has confirmed previous observations in that the coagulase reaction involves the participation of a plasma factor which has heretofore not been described Dried plasma and plasma stored in the frozen state for long periods

tilled water the inhibitor was found to be associated with the insoluble euglobulin fraction while the pseudoglobulin fraction contained little or no inhibitory action

It appeared possible that the presence of this globulin inhibitor in high concentration might be in part responsible for the resistance to the action of coagulase observed in some human plasmas [6] and in the plasmas of certain species such as the guinea pig [3] mouse and fowl [7] Smith and Hale[7] stated that the resistance of these plasmas might be explained by their deficiency of activator The participation of an inhibitor was not recognized

In order to determine the relationship of the inhibitor to coagulase susceptibility tests were carried out for the presence of inhibitor in both susceptible and resistant plasmas Human and rabbit blood was used as a source of susceptible plasma while the resistant plasma was obtained from the guinea pig Previous studies have indicated that of these three species rabbit plasma is the most susceptible [11] Guinea pig plasma is unusual in that it is not clotted at 37 C but is clotted slowly at 25 C [3 7]

TABLE 8 —*Titer of inhibitor in human plasma fractions*

	Plasma Fraction Tested	Dilution of Fraction					
		1	2	4	8	16	32
1	25–60% sat $(NH_4)SO_4$	o	o	o	o	+	+
2	25–40% sat $(NH_4)_2SO_4$	o	o	o	o	+	+
3	40–60% sat $(NH_4)SO_4$	+	+	+	+	+	+
4	60–100% sat $(NH_4)SO$	+	+	+	+	+	+
5	Pseudoglobulin	+	+	+	+	+	+
6	Euglobulin	o	o	o	o	+	+

+ = clot formed o = no clot formed

The susceptibility of each plasma was tested by the routine coagulase test both at 37 C and at 20 C It was found that at 37 C the clotting of human and rabbit plasmas occurred in thirty minutes while clotting of guinea pig plasma occurred in sixty minutes At 20 C the human and rabbit plasmas were clotted in two hours while guinea pig plasma was clotted in eight hours

Thus in this study the effect of temperature was precisely the reverse of that previously reported Guinea pig plasma was definitely clotted by the strain of staphylococcus employed and the clotting occurred more rapidly at 37 C However this plasma was still less susceptible than the plasmas of the other two species

The inhibitor content of each plasma was determined by testing the extracted globulin fraction since it was desired to avoid any possible interference resulting from the presence of activator in the whole plasma Each sample was first defibrinated at 56 C and the globulin precipitate then separated from the resulting sample by half saturation with ammonium sulfate The precipitate was removed by filtration and was dissolved in distilled water and the volume brought up to that of the plasma originally used Each preparation was then titered for its inhibitor content

HEMOPHILIA A CLINICAL STUDY OF FORTY PATIENTS

By Charles S Davidson M D Robert D Epstein M D George F Miller M D and F H L Taylor Ph D

Introduction

SINCE 1803 when hemophilia was first accurately described by Dr John C Otto [1-3] investigators of this disease have centered their efforts chiefly in the elucidation of its hereditary nature and in the study of the constant defect in blood coagulation In the study presented here we wish to emphasize certain clinical manifestations of the disease and methods of practical therapeutic management which have been learned in this laboratory during the last ten years in the course of a study of the defect in coagulation of the blood in individuals with the disease The deep interest of Dr George R Minot in hemophilia began in 1916 when he and Dr Roger I Lee first demonstrated in this country that whole blood transfusions were effective in shortening the blood coagulation time [4] He has been the guiding spirit of the investigative work in hemophilia in this laboratory and this presentation is dedicated to him His guidance in this problem has given experience to many young men in the methods of clinical investigation

Hemophilia is an hereditary disease limited to males those afflicted exhibiting both impaired coagulability of the blood and a strong tendency to bleed especially following trauma Although there may be variations in the frequency and severity of hemorrhagic episodes the disease is always present for life Transmission of the disease is always through the female to the second generation the genes being sex linked and recessive Although the possibilities exist of both a first generation male with hemophilia as well as a female with the disease authenticated cases are not known

Although Otto was the first to bring the true nature of the disease into clear focus there is evidence that certain aspects had been known in ancient times by the Arabs and the Jews Bullock and Fildes[5] in their classical monograph on the disease report descriptions of a condition resembling hemophilia by Albucasis in the tenth century Among the recent general articles or monographs on hemophilia are those of Birch [6] Howell [7] Stetson and Lozner [8] Quick [9] Davidson and McQuarrie [10] Ely [11] Mills [12] MacFarlane [13] and Kark [14]

The coagulation defect in hemophilia is observed in vitro as a prolongation of the whole blood clotting time Normal blood clots in from 6 to 12 minutes as measured by a modification[15] of the method of Lee and White [16] The blood of a patient with hemophilia under the same circumstances may not clot for many hours However most of the patients in our series have clotting times of from 1 to 2 hours but in a few the clotting time is in the range of 20 to 40 minutes Al

From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) and the Department of Medicine Harvard Medical School Boston Mass

This study was aided in part by a grant from the Smith Kline and French Laboratories Philadelphia Pa

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though it has been reported[6] that patients with hemophilia will occasionally exhibit a normal coagulation time this phenomenon has never been observed in this laboratory

The coagulation defect in hemophilia has been ascribed by some to an abnormality of the platelets[4] and by others to the presence of an antithrombin [17] However hemophilic blood will coagulate promptly upon the addition of thrombin Tocantins has described the presence of an antithromboplastin in hemophilic blood [19] while workers in this laboratory believe that there is a deficiency of some factor associated with plasma globulins [18] Nevertheless it is generally agreed that fibrinogen prothrombin calcium and the *number* of platelets are all normal in hemophilic blood Thus whatever the abnormality for practical purposes transfusion of whole blood or plasma and certain plasma derivatives will usually bring the blood coagulation time to or near to normal Hemophiliacs have been observed [0-2] whose coagulation time does not respond as is customary to the administration of blood plasma or its derivatives The basis for this failure to react has not been fully elucidated

Fractionation of blood plasma has led to the identification of the antihemophilic *activity with the euglobulins*[15, 23, 4, 25] and particularly *with fraction* I [6] according to the nomenclature used by Cohn et al Fraction III 2 also contains considerable antihemophilic activity Because of the impracticability at present of the administration of fraction III 2 the fibrinogen fraction I has been chiefly studied in vivo [7] and contains antihemophilic activity which has been clearly shown not to be fibrinogen itself [8, 29]

Occasionally patients are seen who have suffered hemorrhagic episodes but whose coagulation time is only slightly prolonged It is very difficult either to establish or exclude the diagnosis of hemophilia in these patients particularly if a family history of the disease is not obtained as is frequently the case Certain laboratory procedures may be helpful in this regard Among these is the well established reduction in the clotting time of hemophilic blood by the addition of small amounts of normal plasma or its derivatives [26] The measurement of the recalcification time of plasma centrifuged at different speeds[31] may prove to be a valuable diagnostic test if the reported results substantiated

Clinical Manifestations of Hemophilia

It is our purpose to report observations on the clinical manifestations and practical management of forty patients with hemophilia all of 12 years of age or over who have been followed in the Thorndike Memorial Laboratory during the last ten years All were males the youngest 12 the oldest 58 Eight were in the second decade of life 19 in the third 7 in the fourth 5 in the fifth and one in the sixth

Twenty eight of the 40 (70 per cent of the series) had a family history of hemo-

philia Twenty five had a known member of the family in the same generation with the disease 14 one generation back 4 two generations while none was able to trace the disease further The lack of a positive family history is in part due to inadequate knowledge on the part of the patients about their families

There were three patients in whom the family history was known and in whose family no hemophilia had appeared during three previous generations Whether these instances represent sporadic hemophilia or whether the disease was carried by the female through successive generations without manifestation in a male offspring is not known but the latter possibility would appear to have more support from the literature

Spontaneous hemophilia has been reported the most recent article by Boggs[3] reviews the reported cases and presents six brothers with the disease whose family history gave no evidence of bleeders although it was known for four generations on the mother s side Boggs admits that the legitimacy of the mother could be questioned The statistical likelihood of the occurrence of hemophilia and of carriers in families has been studied by Haldane and Philip[33] who have said the daughters of hemophilic men bear equal numbers of normal and hemophilic sons whilst half the sisters of hemophilic men are heterozygous for hemophilia The number of individuals in the two sexes in hemophilia was shown to be normal by Macklin[34]

Most of our cases were of recent European extraction The family extractions (known in 38 of the families) were New England 6 Nova Scotia 7 Irish 8 Italian 7 Jewish 3 English 2 Eastern European 5 There were no Orientals or Negroes in the present series although hemophilia has been reported in both mixed and presumably full blooded Negroes[35 36 37 38] and six probably authentic cases have been described in native Japanese[39] Ten of the patients in this series are married with a total of 13 children 3 males and 10 females There are no grand children

There were five deaths in the series of forty patients in ten years Three of these were from conditions quite unrelated to hemophilia one age 16 from fractured skull and broken leg following an automobile accident the second age 32 from cerebral hemorrhage in terminal malignant hypertension and the third age 34 from pulmonary tuberculosis The fourth age 21 developed an apparently spontaneous massive hematoma in the left gluteal and thigh muscles with secondary necrosis slough and sepsis The fifth death was from rapid submucosal pharyngeal and laryngeal hematoma formation which blocked the airway before help was available

There were no deaths in this series from acute blood loss the popularly supposed cause of death in hemophilia This was in spite of frequent tooth extractions and five relatively serious operative procedures (cf section on Treatment Surgery in Hemophilia) Moreover most of the patients at some time have been admitted to the hospital with a severe hemorrhagic episode

First Hemorrhagic Episode

In 36 of the patients the first hemorrhagic episode was known and varied in time of onset from the age of one week to 13 years Three were following circumcision in the first two months (two at the age of one week) Eight others had their first bleeding during the first year of life two developed an hematoma of the head from known trauma two bled from cut lips one had an hemarthrosis of the knee one hematoma around the knees from crawling one multiple hematomata and for one the precise nature of the bleeding had been forgotten The remaining 25 patients experienced their first hemorrhagic episode during childhood 19 before five years of age having a variety of hemorrhagic lesions not differing essentially from those to be described for adult life and in most following known trauma

Excessive bleeding from primary dentition occurred in only one instance of the 22 in whom the history was available while 13 of 22 had excessive bleeding from secondary dentition Hemorrhage following the extraction of permanent teeth is much more frequent and will be discussed in a separate section

Hemarthrosis

Bleeding into joints is the most frequent hemorrhagic episode in adult hemophiliacs It is usually repeated often so that eventually many joints acquire some degree of permanent damage Thirty six of the patients in this series had chronic hemophilic joint disease and almost all of these gave a history of one or more acute hemarthroses Of the four who exhibited no chronic joint disease and had no history of acute hemarthroses two had suffered relatively few hemorrhagic episodes of any kind

Acute hemarthroses and chronic hemophilic joint disease affected the joints in about the same incidence the knees and elbows being by far the most frequently involved The ankles hips and shoulders were affected much less frequently and the wrists fingers and toes only occasionally

Acute hemarthroses frequently occur without known external trauma although joints especially those bearing weight are subject to the continual trauma of movement The hemarthrosis is heralded by stiffness that soon becomes painful on movement of the joint It is followed within a few hours by swelling which gradually distends the joint capsule causing severe pain even at rest being greatly aggravated by motion Tenderness is exquisite and limited at least at first as is the swelling to the areas where the joint surface is relatively superficial For example in the elbows the areas lateral to the olecranon are swollen tense and exquisitely tender The blood may break through the tense capsule and be released into the neighboring tissues temporarily relieving somewhat the pain and tenderness of the hemarthrosis When this occurs the blood may dissect superficially giving the typical discoloration of an ecchymosis However usually the blood remains confined to the joint and discoloration is then not observed It is because of this lack of discoloration around a joint that an acute hemarthrosis is sometimes mistaken for other forms of acute arthritis During the acute phase the joint is usually held in the position of greatest relaxation the knees and elbows for

example in partial flexion and any attempt to change the position is attended by severe pain.

Usually in from four to six days recovery from the acute phase begins. Pain and tenderness subside a little and the previously tense stretched skin over the joint shows a fine wrinkling. Recovery usually then proceeds rapidly but may require two or three weeks before it is maximal. Residual limitation of motion is common and may become permanent, particularly if the joint has been the object of frequent previous attacks.

Acute hemarthrosis has been mistaken for acute rheumatic fever, rheumatoid, gonococcal and other types of arthritis but may be readily differentiated if hemophilia is considered.

Chronic Hemophilic Joint Disease

Following repeated acute hemarthroses a chronic and often deforming joint disturbance occurs. This is not to be thought of as a chronic hemarthrosis but rather as the result of frequent irritation to the joint leading to roughening of the joint surfaces and fibrosis together with both areas of bone reabsorption and new bone formation. The description of both acute and chronic hemophilic joint disease by Konig[40] is the classical one, but a considerable body of literature has been published on the subject. Caffey and Schlesinger[41] point out that coxa plana resembling Perthe's disease may be the result of joint hemorrhage and further that epiphyseal overgrowth and precocious ossification may be demonstrated by x ray. Fonio,[42] Newcomer,[43] Lamy,[44] Keifer and Myers,[45] and MacDonald and Lozner[46] have discussed the clinical and x ray findings. The latter two papers are based on patients included in the series reported here.

In spite of active preventive measures, such chronically affected joints usually show some limitation of motion and may eventually become ankylosed. The joints are enlarged, the characteristic fusiform appearance being accentuated by atrophy of muscles on either side of the joint. Tenderness and pain on movement are not characteristic of chronic hemophilic arthritis; in fact, if either is present, recent active bleeding has probably occurred.

In only two of this series of forty hemophiliacs was there no evidence of chronic hemophilic arthritis. One would, in fact, hesitate to make the diagnosis of hemophilia without the presence of joint deformity unless the diagnosis could be otherwise conclusively established.

Arising usually after extensive bleeding into and around a joint, Volkmann's contracture sometimes becomes a serious deformity, greatly limiting usefulness of the extremity.[47–50]

Hemorrhage into the Skin, Subcutaneous Tissue and Muscles

Purpura is not the characteristic phenomenon in hemophilia that it is in purpura hemorrhagica. Ecchymosis and hematomata when they occur usually follow known trauma rather than appear spontaneously as they do in purpura hemorrhagica. Ecchymoses seldom spread extensively but hematomata into subcutaneous tissue often spread until they are limited by fascial attachments.

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Bleeding into joints is the most frequent hemorrhagic episode in adult hemophiliacs It is usually repeated often so that eventually many joints acquire some degree of permanent damage Thirty six of the patients in this series had chronic hemophilic joint disease and almost all of these gave a history of one or more acute hemarthroses Of the four who exhibited no chronic joint disease and had no history of acute hemarthroses two had suffered relatively few hemorrhagic episodes of any kind

Acute hemarthroses and chronic hemophilic joint disease affected the joints in about the same incidence the knees and elbows being by far the most frequently involved The ankles hips and shoulders were affected much less frequently and the wrists fingers and toes only occasionally

Acute hemarthroses frequently occur without known external trauma although joints especially those bearing weight are subject to the continual trauma of movement The hemarthrosis is heralded by stiffness that soon becomes painful on movement of the joint It is followed within a few hours by swelling which gradually distends the joint capsule causing severe pain even at rest being greatly aggravated by motion Tenderness is exquisite and limited at least at first as is the swelling to the areas where the joint surface is relatively superficial For example in the elbows the areas lateral to the olecranon are swollen tense and exquisitely tender The blood may break through the tense capsule and be released into the neighboring tissues temporarily relieving somewhat the pain and tenderness of the hemarthrosis When this occurs the blood may dissect superficially giving the typical discoloration of an ecchymosis However usually the blood remains confined to the joint and discoloration is then not observed It is because of this lack of discoloration around a joint that an acute hemarthrosis is sometimes mistaken for other forms of acute arthritis During the acute phase the joint is usually held in the position of greatest relaxation the knees and elbows for

clot formation Generally the pain is typical renal colic indicating that the bleeding is from the kidney or pelvis Following a bout of pain a clot is sometimes passed during micturition and is usually accompanied by severe dysuria

An attack of hematuria may last a day or so or may be prolonged for several weeks no known factor or form of treatment apparently affecting the duration

Initial or terminal hematuria are seen occasionally as a complication of disease in the anterior or posterior urethra The spontaneous hematuria of hemophilia may be confused with any one of the other causes of the symptom for which investigation should be made particularly if the hematuria frequently recurs Weil[54] believes that hematuria in hemophilia is in most instances caused by the presence of stone The frequent occurrence of hematuria in the disease makes this appear unlikely

Although the urine may become quite dark the actual loss of blood during an attack of hematuria is usually not enough to alter significantly the blood hemoglobin content

Pharyngeal and Laryngeal Hematomata

Hematoma formation beneath the mucosa of the pharynx and larynx is one of the few emergencies in hemophilia because of the rapid occurrence in some of airway obstruction Fortunately this does not occur very often although one of the five deaths in this series was from this cause Baird and Fox[55] found seven instances of this complication reported in four of whom tracheotomy was not performed and who recovered while three died following this operation In their own case tracheotomy was done with recovery

The patient usually complains first of sore throat loss of voice or both With either of these symptoms careful examination of the larynx and pharynx must be made at once Sometimes the bleeding begins as an obvious swelling on the posterior pharyngeal wall but more commonly it cannot be seen except by indirect laryngoscopy In the latter case the hemorrhage frequently discolors the mucous membrane over the arytenoids and spreads down the laryngeal wall to the false and true cords Fortunately obstructive symptoms have not occurred in most of our patients but when they do they may either appear within a few hours or be delayed for a day or so

It has been our custom to hospitalize each of these patients at once and to keep a tracheotomy kit readily available (see section on Treatment)

Although most instances of pharyngeal and laryngeal hematoma formation appear to be spontaneous it may follow overvigorous use of the voice against which hemophiliacs should be cautioned

Pulmonary and Pleural Complications

Pulmonary and pleural bleeding are uncommon complications in hemophilia although mediastinal and pleural shadows appearing in roentgenograms presumably from fresh or old hematomata have been reported [56] Massive hemothorax and hemoptysis are rare [57] These complications were not observed in our patients

Bleeding into muscle almost always follows severe trauma and may spread rapidly usually into the subcutaneous tissue and along fascial planes Subcutaneous and intramuscular hematomata are usually much larger than superficial examination would suggest Shock from blood loss is not uncommon and anemia icterus (with an increased indirect serum van den Bergh reaction) reticulocytosis and urobilinogenuria follow Hemorrhage into the gluteal region with spread into the thigh is one of the most common and because of the amount of available space may be extensive and lead to early shock

Hemophilic Pseudo Tumor

Occasionally bleeding into or around bone tissue may be extensive and persistent enough to interfere with the blood supply and cause reabsorption of bone This is observed chiefly in the hands or feet and the part may be converted in the course of weeks or months into a swollen tense sac of old blood and destroyed tissue X ray examination is usually misinterpreted as sarcoma because of the soft tissue swelling and bone absorption Firor and Woodhall[51] reviewed the literature on this subject and reported a case of their own a 15 year old boy who developed a gradually progressive swelling of the right thumb over 18 months following injury X ray revealed absorption of bone and a diagnosis of bone sarcoma was made Successful amputation was done with the aid of an electric cautery A 16-year old boy in our series had a similar occurrence which developed over the course of almost a year and involved the left foot from the mid tarsus distally The metatarsal bones were almost completely resorbed and an x ray diagnosis of sarcoma was made Surgical amputation was done with great care and with a good result

In addition to the pseudo tumor of the distal end of the extremities other changes such as calcification in a subperiosteal hematoma have been described as sarcoma In these instances there may be reabsorption of bone also making the resemblance to sarcoma of bone the more real Starker[5] discussed subperiosteal hematoma in hemophilia and Echtermacht[53] described a 13 year old boy with a huge hematoma associated with the left tibia that was mistaken at first for tumor The patient died three days after amputation

Hematuria

Attacks of hematuria are one of the most frequent hemorrhagic episodes in hemophilia in fact almost 90 per cent of the patients in our series have had one or more episodes Recurrent attacks are very common The attacks are usually spontaneous but occasionally follow direct trauma to the kidney region In one instance an attack was apparently induced by a prolonged train ride the patient being frequently jarred while sitting up in the coach

The onset of hematuria is usually symptomless except for the appearance of red urine Occasionally pain may herald the beginning of the attack or may occur at any time during the course but it is most common toward the end The pain is due to the passage of clots and its location and character depend upon the site of

bowel obstruction vomiting cramplike abdominal pain and abdominal distention A tender low intra abdominal hematoma usually forms after a day or so and finally after several days it may discharge its contents into the bowel with the sudden apearance of melena Patients exhibiting this condition have been described by Vance[59] and Platou and Platou[60]

Retroperitoneal bleeding is more common in the low abdominal syndrome than that associated with the colon and is usually due in these instances to bleeding into or around the ileopsoas muscle The fact that 15 of our 40 patients had at least one episode of ileopsoas hemorrhage illustrates its frequency and importance as a complication of hemophilia The syndrome has been described by Birch[61] Gunther[62] and Fallroth[63] When on the right side the ileopsoas hemorrhage resembles acute appendicitis although the pain seldom begins in the epigastrium At first the pain is mild but usually in the course of hours becomes severe Tenderness to palpation and percussion are often exquisite over McBurney s point and rebound tenderness is the rule There may also be tenderness on rectal examination on the affected side Leukocytosis is almost always present but usually is moderate The blood loss is seldom sufficient to produce anemia or signs of acute blood loss A mass due to a retroperitoneal hematoma often appears within 24 to 48 hours and may be mistaken for an appendiceal abscess even though the latter seldom appears this early after the onset of the symptoms Occasionally the hematoma spreads distally down the ileopsoas muscle and may become palpable at Poupart s ligament or even in the femoral canal When this occurs differentiation from acute appendicitis becomes easier

Further aid in differentiating ileopsoas hemorrhage from other intra abdominal conditions is the distressing complication of partial or complete involvement of the femoral nerve This usually begins with pain on the anterior surface of the thigh and may be observed soon after the onset of the bleeding A positive psoas sign may be seen at this time and the hip is usually held in partial flexion Paresthesiae and usually partial or complete anesthesis often follows within two or three days and weakness or paralysis of the thigh extensors with subsequent muscular atrophy follows As mentioned above the acute episode lasts as a rule for but a few days but the mass when present may disappear slowly or even may remain permanently Likewise the femoral nerve damage is slow to heal and hypesthesia muscular weakness and atrophy may be permanent

Neurologic Complications in Hemophilia

Spontaneous intracranial hemorrhage is rare in hemophilia[63] in contradistinction to purpura hemorrhagica in which it is the most common cause of death[9] Bleeding into or around the spinal cord is likewise seldom seen in hemophilia although retroperitoneal hemorrhage sometimes impinges upon a nerve root as it emerges from the spine producing typical unilateral radicular pain

Peripheral nerve lesions of varying severity and location are very common and usually complicate hemorrhage into a joint or muscle which is in close proximity to the nerve Thus the ulnar and superficial peroneal nerves are frequently damaged

The Acute Abdomen in Hemophilia

Not only are the usual acute abdominal conditions a problem in hemophilia because of the high operative mortality [58] but in addition certain forms of intra abdominal and retroperitoneal hemorrhage so resemble acute surgical emergencies that the greatest diagnostic acumen and surgical caution must be exercised to avoid a fatal result

All the common acute abdominal conditions such as acute appendicitis acute cholecystitis perforated peptic ulcer acute pancreatitis etc may of course appear in hemophiliacs Although it is difficult to ascertain the degree bleeding from or into the damaged tissue may complicate these acute abdominal conditions by increasing the symptoms and delaying healing Where infection is present it may travel with the bleeding and in this way spread much farther than it otherwise would Therapeutic procedures will be discussed in the section on treatment

Hemophiliacs in addition suffer a variety of purely hemorrhagic intra abdominal episodes which both closely mimic and are more frequent than the usual acute abdominal emergencies In many instances such hemorrhagic episodes are difficult if not impossible to differentiate from the common forms of the acute abdomen Sometimes the course of the illness establishes whether it is purely hemorrhagic or not but all too frequently the differentiation is obscure and it is extremely difficult to decide not to perform a highly dangerous operation

Severe upper abdominal pain usually cramp like but sometimes steady and resembling a penetrating or even perforated ulcer is occasionally seen The onset is usually progressive over several hours with pain reaching great severity and usually associated with nausea and vomiting The abdomen may become distended with upper abdominal tenderness or even generalized tenderness and a board like rigidity Moderate leukocytosis is usual The acute condition usually lasts from one to two days and then gradually subsides over a period of several days or occasionally recurs To place the bleeding accurately in these episodes is usually difficult In some instances intraperitoneal bleeding becomes evident by the appearance of free fluid in the peritoneal cavity together with signs of acute blood loss A positive benzidine or guaiac reaction in the stool a day or so after the beginning of the episode indicates bleeding into the gastro intestinal tract which may be due only to mucous membrane bleeding from persistent retching and vomiting Massive melena may sometimes complicate this upper abdominal bleeding syndrome but hematemesis is rare

Pain in the midabdomen usually cramplike and resembling small bowel obstruction is a distressing although uncommon complication in hemophilia and is probably due in most instances to bleeding into the bowel wall the mesentery or both and sometimes associated with intra abdominal bleeding Moderate distention and vomiting are the rule and are due to paralytic ileus

Low abdominal pain is the commonest of the abdominal emergencies in hemophilia Two apparently unrelated forms of bleeding may occur into the colon wall or the mesocolon or into or around the ileopsoas muscle In the first instance bleeding into the colonic wall or mesentery the signs are usually those of partial

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in this way Retroperitoneal ileopsoas hemorrhage affecting the femoral nerve is discussed above in the section on the acute abdomen A very complete review of the neurologic complications of hemophilia is to be found in an article by Aggeler and Lucia [65]

Therapy in Hemophilia

In addition to the many manifestations of hemorrhage itself bleeding in hemophilia may complicate other coexistent diseases The treatment of these primarily nonhemorrhagic conditions may be further complicated by secondary hemorrhage Treatment is directed both to rectifying the diminished blood coagulability locally and systemically as well as to whatever nonhemorrhagic condition may be present

1 *Blood coagulants* For generations man has been searching for methods to stop bleeding and the number of remedies both household and medical, attest both to the frequency of the problem and the general inefficacy of the methods of hemostasis In an effort to halt the excessive bleeding in hemophilia a great many remedies have been described most being for parenteral administration [66–79] We have had little or no experience with most of these therapeutic agents many of which have been proven ineffective Since Veil [80] in 1905 found that the therapeutic effect of blood transfusions in hemophilia was due to bringing the coagulation time to or near normal this form of therapy has not only passed the test of time [4, 81, 8] but also is the most physiologic of all the parenteral remedies tried However even when the coagulation time is brought to normal with blood transfusions the bleeding may continue

Since the antihemophilic activity of both blood and plasma gradually disappears when preserved even at refrigerator temperatures [83] it has been our policy to use human whole blood or plasma not over 24 hours old unless in the case of plasma it has been separated soon after phlebotomy and preserved in the frozen state Lyophylized plasma has been shown to be active [84] but for optimum effectiveness it can not always be depended upon as several days often elapse between the drawing of the blood and its processing

In the case of acute blood loss of significant proportions either externally or into the tissues fresh whole blood is the choice for it not only provides antihemophilic activity but replaces the loss in both red cell and plasma volume Plasma fresh or frozen is simpler because cross matching is not required and it is as rich as whole blood in antihemophilic activity It has been our custom to administer whole blood in the amounts dictated by the severity of the blood loss If whole blood is not necessary plasma is given in 100 cc to 250 cc quantities for its antihemophilic properties The reduction in coagulation time is usually to or near to normal This effect persists for 6 to 12 hours at the minimum and then the clotting time gradually rises to its preinjection level in the course of another 6 to 12 hours [7] (fig 1) Thus for continued effect on the coagulation time of the hemophilic patient blood or its products should be given once or perhaps twice daily during the period of active bleeding

A hemophilic may vary considerably from time to time in his response to antihemophilic material of known potency It is important to determine the coagula

tion time shortly after the administration of the antihemophilic agent e g ½ hour and again at a 6- to 8 hour interval in order to follow the extent and duration of the effect If the coagulation time does not reach or remain at or near normal the administration should be repeated

As described above in the section on the coagulation defect in hemophilia blood plasma fractionation has led to the production of a preparation of human fibrinogen which contains antihemophilic activity and which can be given intravenously to patients with hemophilia [7] In the dosage recommended there have been no *significant reactions observed and no reported cases of serum jaundice have oc*curred * In addition to absence of icterogenic properties the material has the ad

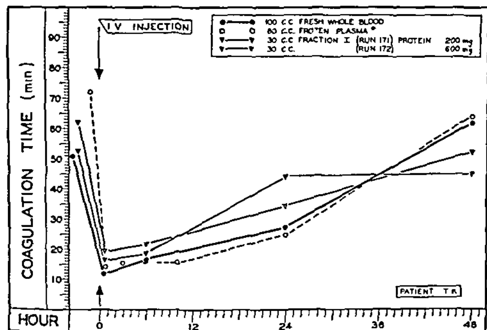


FIG 1

vantages over whole blood and blood plasma that very small amounts need be administered for maximum effect and that it can be easily and quickly given There is a great deal of variability in the antihemophilic activity of Fraction I as now available and there are in fact instances when fresh whole blood is more effective in reducing the coagulation time Thus the material is in no sense a cure for hemophilia but its production is a step toward finding a potent therapeutic substance and hopefully a prophylactic material which could be given in hemophilia much as insulin is to a diabetic For many years such a preparation has been the dream of both hemophiliacs and investigators In his lectures to students Dr Minot has often referred to this goal At present the limitations in the avail

* Since this paper has been submitted for publication two cases of hepatitis probably transmitted with the administration of Fraction I have been observed

able quantity of Fraction I and problems of stability and route of injection have prevented its use as a prophylactic Nevertheless, attempts at maintenance of a reduced blood coagulation time have been made by injecting fresh[85] or lyophylized[14] plasma once a week or more often Significant prolongation of the interval between hemorrhage has been obtained in this way

The refractory state to blood and its derivatives referred to in the introduction *follows in some instances* the repeated administration of blood plasma or the antihemophilic globulin fraction and arises during or promptly after an hemorrhagic episode although occasionally it is spontaneous * The exact nature of this refractory state is still obscure, but recent work has suggested that there may be a production of antibodies to the antihemophilic substance [21] This observation has yet to be confirmed

2 *Rest and exercise* Although strict precautions must be taken by the hemophiliac against trauma this does not mean that he should live a sheltered inactive life Heavy manual labor prolonged fatiguing exercise the more vigorous sports and other activities that require severe physical exertion should not be attempted however moderate activity should be encouraged depending upon the physical capabilities of the individual *for it not only gives the individual a sense of equality* with his associates but also helps to maintain muscle tone and joint mobility It is our impression that the decrease in muscle size and tone which occurs with immobilization and disuse[86] may be an important factor in initiating hemorrhage into the muscles and neighboring tissues Although it is difficult to evaluate because of the possible cyclic frequency of hemorrhages bed rest with its attendant inactivity appears to us to be an important predisposing factor to hemorrhage Thus a hemophiliac confined to bed for an acute hemarthrosis for example not uncommonly develops hemorrhage in other parts of the body Convalescent patients are therefore encouraged to take moderate exercise The aid of an expert physical therapist should be available for directing exercise both while the patient is in bed and during ambulatory convalescence

3 *Use of sedatives and analgesics* The fact that internal hemorrhage in hemophilia is regularly accompanied by severe pain which may last for several days or more and that repeated episodes may be expected throughout the patient s life makes the choice and use of analgesics difficult and of prime importance The use of morphine is sometimes necessary but should be avoided if possible If it is required the drug should be administered for as short a period as possible because of the danger of dependence and habituation Meperidine hydrochloride (demerol hydrochloride) also contributing to addiction [87] has been useful in our hands but occasions arise in which only morphine is effective When it is decided to administer these or similar analgesics maximum effective doses should be used to control the pain

Aspirin often fortified with codeine is often effective for less severe pain but *in the case of codeine too care against habituation* must be taken since moderate pain may be prolonged for weeks as for example following an hemarthrosis Hypnosis with barbiturates may make pain bearable especially at night

Presently available evidence suggests that this refractory state may occur more freq ently follow ing the *administration of the antihemoph l c* globulin fraction than follo ving the administration of blood or blood plasma The therapeutic se f the antihemophilic globulin fraction cannot be ad ised therefore until further studies have eliminated this hazard

4 *Local treatment of external bleeding* Aside from the parenteral administration of blood and its derivatives to reduce the blood coagulation time many substances have been produced for use locally at the site of bleeding Some of these preparations are very poor coagulants and most of those that are effective at all exert their effect as a thromboplastin That is they hasten the coagulation of the blood by action with prothrombin and calcium resulting in the production of thrombin which converts fibrinogen to fibrin We prefer thrombin as a coagulant because it directly *converts the fibrinogen to form a fibrin clot We have had excellent re*sults with a thrombin prepared from animal blood *[55] [59] Thrombin may also be prepared from human blood[59] and has recently been produced on a large scale as a by product of the preparation of human serum albumin from plasma [90]

No matter what local coagulant is chosen adherence should be made to certain general principles The wound should be cleaned with as little trauma as possible debris and clots of blood being gently removed Approximation of the edges may be desired but should not be made with sutures unless absolutely necessary as each needle hole is another source of bleeding Thrombin is applied directly to the site of bleeding and is held there by appropriate pressure dressings It is important to emphasize that the thrombin must be applied directly to the source of bleeding if not it will merely form a blood clot in the wound keeping it open and preventing approximation of the edges effective hemostasis and healing The two principles of treating superficial wounds in hemophilia then are first that a known active coagulant be applied to the bleeding surface and second that it be maintained there with some form of pressure dressing

Some of the earliest surgical experiences with hemophiliacs were with the use of cautery both chemical and thermal Poland[46] in 1850 described a patient in whom pure nitric acid stopped bleeding from a traumatic lip lesion on two occasions Ericksen[91] in 1856 tells of a 34 year old male who developed an hematoma extending from the ankle to the popliteal space Following incision bleeding areas were touched with cautery with cessation of bleeding Gangrene developed however and following amputation by ligature and cautery the patient died

Although cautery may temporarily stop bleeding its use is not advised since surrounding tissues are usually destroyed or damaged leading to a secondary area of slough and an enlarged area of bleeding Thus a ten year old boy seen by one of us had been treated by cautery with dichromate for a clean tongue cut The bleeding stopped only to recur two days later with renewed vigor from a larger wound this time being stopped only by the application of thrombin on a gauze pack *held in* place by sponge forceps

5 *Surgery in Hemophilia* Operative surgery in patients with hemophilia is hazardous and attended by a high mortality [9] Friedrich[58] estimated a 35 per cent mortality following major operations and his estimate is probably a conservative one The operative treatment of specific conditions will be discussed in subsequent sections

Medical literature concerning hemophilia is replete with reports of various surgical procedures which have been attempted In most instances some form of local or parenteral coagulation therapy was used often in addition to blood trans

Hemostatic Globulin (Dried) furnished by Lederle Laboratories Division American Cyanamid Company Pearl River New York

fusions Many of these were listed above (Section 1 Blood Coagulants) but are not discussed because of their large number and variety and the lack of precise observations of their effectiveness Some have been shown to be ineffective

In spite of the high mortality rate operations sometimes of considerable magnitude have been done Among those reported some of which were successful but many not are appendectomy[93, 94] gastro enterostomy [96] partial gastrectomy [97] arthroplasty [98, 99] eye enucleation [100] prostatectomy [101] nephrectomy [10] mastectomy [59] and various amputations [103]

If an operation is decided upon the free use of preoperative and postoperative blood transfusions and, when possible the local application of thrombin (Section 4) are the only important additions to careful surgical technics Specific surgical problems will be discussed as they occur in the following sections

6 *Treatment of acute hemarthrosis* When an acute hemarthrosis occurs in the lower extremity bed rest is necessary otherwise the patient may be ambulatory with a sling or other support if the pain permits Ice bags to the part give some symptomatic relief Pain is usually extreme and analgesia is indicated Compression bandages applied before much swelling has occurred have been found useful by some Aspiration of the fluid blood in the joint is not recommended because of the danger of infection and moreover in our hands has failed to shorten convalescence significantly Following aspiration of blood the joint pain is usually greatly relieved but returns again in a very few hours Thrombin preparations (sterile human) may be injected into an acute hemarthrosis but this therapy has not yet proven to be of value

It is usually not possible to place the affected joint in optimum functional position during the acute phase nor do we consider it necessary since as soon as signs of reabsorption of blood appear cautious active movement up to the point of pain may be begun and gradually increased usually until the former range of movement is attained As convalescence progresses and danger from renewed bleeding diminishes physical therapy is in the form of radiant heat and whirlpool baths hasten recovery of function Early active movement and physical therapy are the best preventatives of ankylosis

7 *Treatment of Chronic Hemophilic Arthritis* Treatment of arthrosed or otherwise deformed joints is largely orthopedic and must be undertaken with great care so that hemorrhage is not induced either into the affected joint or at points of pressure The use of plaster casts which are gradually wedged to the desired position has often been successful The amount and frequency of the wedging is distinctly less than in nonhemophilic patients each spreading of the cast being up to the point of first pain Simple Buck s extension is also frequently useful but the same precautions must be observed

Arthroplasty like other operative procedures in hemophilia must be seldom undertaken and then only with full knowledge of the mortality as well as the likelihood of a poor result from bleeding into and around the operative site If operation is decided upon the suggestions listed under Surgical Treatment may be helpful in avoiding complications

However in spite of these measures the joints of patients with hemophilia may

become partially or completely ankylosed with deforming muscle contractures and atrophy When this happens in the legs symptomatic calluses usually develop on the feet Softening and removing these calluses provides only temporary relief but more prolonged help can be obtained with corrective shoes Patients with hemophilia can use aids to walking without difficulty such as canes and crutches and we have one individual in our series who is successfully wearing a prosthetic for a surgically amputated foot

8 *Treatment of subcutaneous and intramuscular bleeding and of pseudo tumor* Bed rest with immobilization of the part is usually automatically resorted to by a patient with a large hematoma of the soft tissues Ice bags as in acute hemarthrosis provide some relief and analgesia is often required Firm pressure from an elastic bandage over the entire area and especially over the bleeding point if known may reduce the bleeding It cannot be overemphasized that a large amount of blood may be lost into the soft tissues without producing what would seem to be commensurate swelling A continual watch of pulse blood pressure and hematocrit must be made so that shock does not occur Blood transfusions not only supply the antihemophilic factor but also replace blood lost

Great care must be taken to prevent ulceration of the skin over the hematoma as infection and renewed bleeding may become major therapeutic problems [104, 105]

Hemophilic pseudo tumor with necrosis and readsorption of bone as well as soft tissues is a potential hazard when fully developed because of its awkwardness and susceptibility to infection Amputations have been done for this condition If undertaken extreme care must be exerted to see that the blood coagulation time is as close to normal as may be obtained and that the surgery induces the least possible trauma Thrombin should be placed between the stump and its covering

9 *Treatment of peripheral nerve lesions* Little further than the treatment outlined in section 7 and 8 can be done to treat the neuritis that not uncommonly develops during the active phase of intramuscular or subcutaneous hemorrhage Complete regeneration of nerves may be expected in the course of time in many instances while some will be left with residual nerve damage Physical therapy to maintain muscle tone and prevent contracture and bony ankylosis of joints is indicated When splints are applied to avoid contracture they should be bivalved so that physical therapy may be instituted

10 *Treatment of hematuria and certain urologic complications* Bleeding from the genito-urinary tract is usually renal in origin and is frequently resistant to treatment continuing in spite of the repeated administration of fresh blood or its derivatives and satisfactory reduction of the blood coagulation time Absolute bed rest in the supine position may be tried but in our hands has been largely ineffectual In occasional patients there may be prompt cessation of bleeding following some form of therapy but generally after a variable period it ceases spontaneously Except for the occasional development of a mild blood loss anemia there have been no ill effects from continued hematuria The ureteral passage of blood clots particularly frequent when bleeding is decreasing usually causes severe renal colic and may require the administration of morphine or demerol for relief

As mentioned above search should be made if suspected for stone tuberculosis

malignancy, or other causes of hematuria particularly if repeated episodes of bleed ing occur Cystoscopy may be performed in hemophilia if necessary and if carefully done but ureteral catheterization or retrograde pyelography may induce submuco sal ureteral bleeding and probably should not be performed

Operative intervention in urologic problems in hemophilia is extremely serious Barney[106] in 1933 described a case in which following a necessary suprapubic cys totomy failure to control the bleeding resulted in death Mertz and Meiks[10] re ported a patient who died eight days after a nephrectomy for hydronephrosis in spite of repeated transfusions Hinman [101] however successfully removed a prostate in a 66 year old hemophiliac

11 *Care of the teeth dental extraction* Dental prophylaxis is of paramount impor tance in the care of the hemophiliac It is to the advantage of the patient that he be seen regularly and often by his dentist and that prophylaxis and necessary repair be performed at an early date Cavities can be filled without fear of hemorrhage although care should be taken to avoid undue trauma to the gums

However frequently due to the failure of the patient to seek dental care or reluctance of the dentist to perform the indicated procedures extraction is neces sary In conjunction with the Department of Oral Surgery Boston City Hospital * the method described below has been successfully employed many times in the last five years

The plan involves reduction in blood coagulation time by parenteral fresh blood or suitable derivates and the application of thrombin with pressure to the socket provided by a partial or complete denture [13, 107, 108] By combining these two tech nics we have been able to perform dental extractions in hemophiliacs with a pro gressive reduction in the postoperative bleeding so that at present it is minimal

Before the extraction is performed an impression is taken of the jaw from which the tooth is to be extracted From this a well fitting partial or complete denture is made Its essential features are a labial flange extending from the main body of the denture across the socket from which the tooth is to be removed and two wire clasps one on either side of the denture that serve to secure it firmly in position Approximately a week prior to the operation a thin tightly fitting band of rubber (orthodontia band) is placed about the neck of the tooth to be extracted During the succeeding several days this band progresses along the tooth root partially sep arating it from the adjacent tissues At times the band will progress rapidly along the root so that it may be necessary to use two or three such bands in order to keep the soft tissues from reapproximating to the tooth after the band has passed

An hour or so before the actual extraction the patient is given an amount of antihemophilic globulin sufficient to reduce his coagulation time to 15 minutes *or lower if possible In the event that this material is not available* fresh whole blood frozen plasma or its equivalents in antihemophilic activity may be used Similar amounts of antihemophilic globulin are routinely administered on the first second and third postoperative days

The principles and technic employed are largely the result of the enthusia tic ork of Dr Stephen P Mallett Oral Surgeon in-Chief and his staff particularly Dr Phillip H White We are indebted to them for the details of this presentation which will subsequently be reported in full

In the majority of our cases novocaine has been used as an anesthetic although nitrous oxide oxygen inhalation anesthesia may be safely employed In extractions of the maxillary teeth it has been the practice of the operator to infiltrate with a fine gage needle the tissues at the free cuff margin of the gingivae rather than using the more conventional type of infiltration By so doing the tissues traumatized are localized in one area over which the mechanical pressure of the denture will be applied Mandibular block injections are usually necessary for the removal of teeth from the lower jaw although in this procedure there is danger of causing pharyngeal hematomata

An attempt should be made to extract the tooth with as little trauma as possible On occasions however small lacerations of the gums have occurred and the socket septa have been removed without increased bleeding

After the tooth has been removed the socket may be gently sponged and cleaned Using an empty novocaine carpule dried thrombin is then firmly packed into the defect and buttressed with a more solid mechanical filler An oxidized cellulose preparation* has proven to be very satisfactory for this purpose No attempt is made to suture the gum margins The denture is then inserted into position care being taken to see that the flange fits firmly over the socket

In the majority of instances there will be insignificant postoperative bleeding If such is the case the denture is not removed for approximately a week At the end of this time it may be taken out for a short trial period If oozing still continues a small amount of dried thrombin is applied to the bleeding surface and the denture reinserted This is repeated at one or two day intervals until complete hemostasis has been obtained If more vigorous bleeding occurs the denture may be easily removed at any time the socket cleaned of old clots and repacked and the denture reinserted

It is of utmost importance to have a well fitting denture It is uncomfortable to the patient if it fits too tightly and local pressure necrosis may occur On the other hand if it fits too loosely sufficient mechanical pressure will not be applied in the appropriate area or the movements of the denture may dislodge the clot and hemostasis will not be obtained By adding flanges as needed to the original denture it may be used for more than one extraction However a new denture has to be made from time to time to compensate for the shrinkage of the soft tissues and readsorption of the underlying bone The unpleasant taste that usually occurs after two or three days wearing of the denture may be partially alleviated with simple mouth washes

During the period that the denture is being worn the patients are permitted to be up and about the ward and to engage in their usual activities They are able to eat and sleep regularly Conventional partial or complete dentures can be worn by the hemophiliac without difficulty

12 *Treatment of pharyngeal and laryngeal hematoma* The potential seriousness of pharyngeal or laryngeal hematoma lies in its occasional propensity rapidly to occlude the airway For this reason if suspected the diagnosis must be confirmed by a completent laryngoscopist and if confirmed the patient should be hospitalized

Oxy-cel provided by Parke Davis & Company Detroit Michigan

so that proper supervision is available A tracheotomy kit is kept near at hand The diet should be soft or liquid and absolute voice rest enforced Administration of fresh blood or its derivatives to reduce the blood coagulation time is essential Generally within 24 hours the swelling begins to recede and convalescence is then rapid and uneventful If obstruction of the airway becomes imminent tracheotomy should be done with the most careful surgical hemostasis and with the liberal use of blood or its derivatives

13 *Abdominal surgery* In the discussion above concerning The Acute Abdomen in Hemophilia the difficulty was emphasized of differentiating either intra abdominal or retroperitoneal hemorrhage from the usual acute abdominal conditions In this regard Traum[7] reported a patient who was operated upon with a mistaken diagnosis of peritonitis from a ruptured appendix An hematoma the size of a child s head was found around the right kidney which was evacuated and packed The patient subsequently died Scherk[109] has discussed the differential diagnosis of abdominal symptoms in hemophilia and described a 47 year old hemophiliac in whom a diagnosis of acute appendicitis was made He was treated without operation in spite of the development of a sausage shaped tumor in the right lower quadrant which disappeared in eight days A gangrenous appendix however was successfully removed by Prima[94] complicated by a fist sized hematoma in the wound Cioran[95] likewise reported the removal of a perforated gangrenous appendix with a good result

It is impossible to be didactic concerning operative intervention on patients with hemophilia in whom an acute abdomen is suspected Two important facts may be reiterated however Intra abdominal or retroperitoneal bleeding is far more common in hemophiliacs than are the usual abdominal emergencies Secondly major surgery has a very high mortality rate in hemophilia With these facts in mind an unnecessary operation usually may be avoided A case in point is that of Platou and Platou[60] concerning an eight year old hemophiliac who was very ill with signs of intestinal obstruction He improved following the institution of continuous gastric aspiration Blood transfusions were administered and operation was delayed from day to day and finally avoided A diagnosis of bleeding into the bowel wall was made *In our experience this set of circumstances has o curred a number of times* and operation has not yet been necessary Likewise in patients with pain in the right lower quadrant resembling appendicitis operation has not been done although their number has been large An ileopsoas hemorrhage was suspected in each In view of the work of Crile[110] with the use of massive doses of penicillin in peritonitis resulting from appendicitis the danger of not removing an acutely inflamed appendix may not be as great as it was formerly considered to be It is probable that occasionally an acute appendix will be missed by this conservative treatment but again the operative risk may be as great or greater than that of an unoperated acutely inflamed appendix

14 *Social economic and psychiatric implications* An hereditary disease with an outlook of life long partial disability inevitably brings with it a multitude of social economic and psychiatric problems It is the physician s duty not only to care for

the hemorrhagic episodes but in addition to consider and advise on such matters as vocation marriage and children

Rightfully preventive therapy must begin in childhood as soon as the diagnosis of hemophilia is established The nature of the disease must be clearly explained to the parents so that they will not only endeavor to prevent hemorrhages but will so orient care for and instruct the child that he will grow into as useful and productive a citizen as possible for only in this way will he be well adjusted and thus happy More than most children he must be taught independence and self reliance and must not depend too much upon his parents This is often difficult for the rest of the family for the hemophiliac is of course subject to frequent bouts of pain which automatically make him the center of attention

Early in life a vocation must be carefully planned Too often hemophiliacs grow to adult life with little formal schooling because of frequent illness Vocational training is likewise scanty so that they are capable only of manual labor for which they are quite unfit A little consideration of the individual and his bent will indicate whether he is to work chiefly with his brain or his hands In the latter category art architecture *mechanical* drawing watch *repairing* electrical and radio work offer opportunities In some communities vocational training of the kind required by hemophiliacs is available

Ten of the 28 hemophiliacs over 20 years of age in our series are married and have a total of 13 children To advise against marriage simply adds another probably unnecessary burden to an already troubled life However one can ensure that both partners understand fully the nature of the disease and their responsibility both as to its hereditary implications and to the prognosis for future morbidity The decision is then left to the individuals concerned It is certainly well for the prospective bride to have a possible gainful vocation in case of prolonged illness of her husband but many of our hemophiliacs have been able by careful planning to provide an adequate home

The hemophiliac is continually exposed from an early age to those who feel sorry for him want to help him or even consider him an inferior *In addition* he has frequent illnesses and must bear considerable pain It requires a strong mental constitution to become adjusted to such a life Fortunately most hemophiliacs accept their additional burdens as they come and in this way each period of stress builds a better adjusted individual The physician by frequent discussions is in a position to aid greatly the individual s own effort to learn to live with his disease

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1 Anticoagulant action of noncitrated plasma on normal whole blood

Blood was obtained from the patient without anticoagulant The red cells were allowed to sediment for 3 hours and the supernatant plasma separated Increasing quantities of this plasma were added to 1 cc of normal human blood Table 1 shows that the patient s blood plasma had a marked anticlotting effect on normal blood

TABLE 1 —*Clotting Time of 1 cc of Normal Blood to Which the Patient s Plasma Was Added (Experiment Done on Nov 14 1946) To 3 C Test Tubes 13 × 110 Duplicate Readings*

Patient plasma (cc)	Normal blood #1 (1 cc) Clotting time (minutes)		Normal Blood #2 (1 cc) Clotting time (minutes)	
0	8	10	10	15
0 1	26	33	45	48
0 2	120	180		

TABLE 2

Patient s citrated plasma (cc)	Normal citrated plasma (cc)	Clotting time (minutes)
0	0 2	4
0 1	2 9	6½
0 1	1 9	17
0 1	0 9	35
0 1	0 4	60
0 1	0	240+

TABLE 3 —*Howell s Time of Patient s Citrated Platelet free and Platelet with Plasma (0 cc plasma plus 0 2 cc $CaCl_2$)*

Date	Number of platelets (per mm 3)	Clotting time (Minutes)	Type of plasma
11-7 46	115 600	32	Platelet rich
	225	360+	Platelet free
11 14 46	125 000	45	Platelet rich
	340	360+	Platelet free

2 Anticoagulant action of citrated plasma from the patient s blood on citrated plasma from normal blood

Citrated normal plasma was obtained by spinning citrated normal blood at 6 000 rpm for 30 minutes Increasing quantities of this plasma were added to a fixed quantity of citrated platelet free plasma from the patient s blood Recalcification of the mixture was done by adding an equal volume of 0 025N $CaCl_2$ and the clotting time (Howell s time) measured Table 2 shows a typical experiment performed on Sept 14 1946 The results can be explained only by the presence of an anticoagulant in the patient s plasma

HEMORRHAGIC DIATHESIS ASSOCIATED WITH THE PRESENCE OF AN ANTICOAGULANT IN CIRCULATING BLOOD CASE REPORT AND LABORATORY STUDIES

By J P Soulier M D and M Burstein M D

THERE ARE three observations of the presence of an anticoagulant in the blood of patients with a hemorrhagic diathesis in the American literature [15 16 4] The following case report constitutes the fourth observation of such an occurrence

The patient was studied at the Hospital des Enfants Malades by Dr Maurice Lamy, under whose direction this work was carried out

M B age 21 male was admitted to the hospital in July 1946 with a diagnosis of hemophilia Past history revealed the frequent occurrence of hemorrhagia episodes the first one occurring at 1½ years of age There were oral nasal and gastro intestinal hemorrhages but most episodes were articular and occurred in the absence of any significant trauma These hemorrhages required blood transfusions on two occasions

Joint hemorrhages were particularly frequent repeating themselves 5 to 6 times in a year They affected mostly the elbows knees and ankle When seen the patient was confined to bed for the last 2 months with involvement of both knees and hematuria

The family history is not contributory He was the only child and his mother died of tuberculosis The maternal grandmother her children and grandchildren were always in good health The paternal grandfather was healthy His father had frequent episodes of epistaxis

Physical examination revealed a pronounced muscular atrophy affecting the inferior limbs mainly a hemophilic arthritis and a malformation of the uvula No hematomas in the muscle The remainder of the physical examination was normal There was no syphilis

The blood coagulation time was 6 hours (at 37 C) the bleeding time 4 minutes The red cells count was 4 1 million with a hemoglobin of 75 per cent

The leukocyte count was 9 800 with the following differential count neutrophiles 76 per cent eo inophiles 2 per cent lymphocyte 16 per cent monocytes 6 per cent

On Aug 2 1946 the patient received a transfusion of 300 cc of blood (fresh) The following day the clotting time was still 6 hour The patient was sent home where he was treated with distilbestrol He was readmitted Oct 31 In the interval period he had no severe hemorrhage episodes There were a few ecchymoses and gingivorhagine The clotting time was 4½ hours and the bleeding time 17 minutes The tourniquet test was negative and the capillary resistance measured by the cuff method was found normal (no petechiae after 6 minutes under a pressure of 90 mm of mercury) Examination of the blood gives the following results Red Cells 5 1 million Hemoglobin 15 g 3 Hematocrit 50 MCV 97 3 µ White Cells 6 400 Platelets 300 000 of which 90 per cent were round forms Fibrinogen 400 mg per 100 cc Calcium 9 9 mg per 100 µ

The purpose of this communication is to report the different studies carried out on the blood of this patient The blood was obtained from the antecubital vein with and without anticoagulant The anticoagulant used was a 4 per cent solution of sodium citrate 1 cc of which was used for every 9 cc of blood Plasma was obtained by centrifuging

From the Centre National de Transfusion Sanguine et de Recherches Hematologiques Paris

This case is the subject of a report under the title Sur un anticoagulant present dans le sang d un sujet se presentant cliniquement comme un hemophile by M Lamy M Burstein and J P Soulier to be published in the Rev d Hematologie

The effect of dilution was studied platelet free citrated plasma from the patient s blood was diluted with saline and then recalcified and the clotting time at 37 C noted

Table 5 shows that dilution shortens the clotting time

4 Effect of concentration of calcium on Howell s time

Lozner Joliffe and Taylor[13] state that the addition of calcium to whole blood obtained from their patient produced a shortening of the clotting time We have not observed any effect in the case reported here from the addition of concentrations of calcium chloride between 0 05N and 0 0125N

5 Effect of storage of plasma on Howell s time

When the patient s citrated plasma was kept at room temperature for 48 hours the Howell s time was not modified provided the plasma was devoid of platelets

6 Effect of normal plasma and fractions of normal plasma on the clotting defect of the patient s plasma

TABLE 5 —*Effect of Dilution on Howell s Time*

Date	Howell s time of undiluted plasma (minutes)	Howell s time of diluted plasma 1 2 (plasma [illegible] 0 9 [illegible] saline) ([illegible] minutes)
7-25-46	360	40
3- 1-46	360	40
11- 7 46	300	90
11-14-46	300	90

We have used normal human plasma and one of the plasma fractions obtained by Cohn and his coworkers in the department of Physical Chemistry of Harvard Medical School * The plasma fraction used was Fraction I of Cohn which in addition to fibrinogen contains most of the antihemophilic activity of normal plasma Two types of fraction I were used One from normal human plasma and the other from hemophilic blood plasma † These fractions were obtained as a dry powder 40 mg of which was dissolved in 2 cc physiologic saline at pH 7 3

Table 6 shows that neither type of fraction I in the quantities used in this experiment had any effect on the clotting defect of the patient s plasma

7 Quick s prothrombin time of the patient s plasma

This was determined by means of brain s thromboplastine the prothrombin time of the patient s plasma determined by this method was 20 seconds very near the prothrombin time of the normal control (table 7) However with smaller amounts of thromboplastin there appears a widening divergence between the patient s plasma and the normal control (table 7)

* Obtained through the courtesy of Dr E J Cohn

† Obtained through the courtesy of Dr F H L Taylor

3 Clotting time on recalcification (Howell's time)

a Effect of platelets on Howell's time

Platelet rich citrated plasma was obtained by spinning citrated blood at 2000 rpm for 3 minutes Platelet free plasma was prepared by spinning the platelet rich plasma at 6000 rpm for 30 minutes and passing the supernate through an Iena ≠4 filter The filtrate was platelet free for all practical purposes Table 3 shows the results obtained by recalcifying both plasmas The Howell's time was considerably prolonged when the platelets were removed Similar results were obtained by Quick with hemophilic plasma It is noticeable that the Howell's time of this patient's plasma devoid of platelets approximated very closely the clotting time of whole blood However the clotting time of platelet rich recalcified citrated plasma was much shorter which may indicate the existence of a destruction of the platelets during recalcification and liberation of thromboplastin from the platelets

b Effect of destruction of platelets on Howell's time

It is well known that the clotting time of citrated recalcified plasma (Howell's

TABLE 4—*Howell's Time before and after Destruction of Platelets*

Date	Clotting time of recalcified citrated platelet rich plasma from patient's blood (Minutes)		
	Intact platelets	Platelets destroyed by freezing	Platelets destroyed by adding distilled water
7-25-46	90	10	15
8- 1-46	90	12	15
8- 3-46	100	20	
11- 7-46	32	20	22
11-14-46	45	13	14

time) is shortened if the platelets are destroyed and allowed to release their thromboplastin—according to Tzanek and Burstein citrated plasma clots faster if before recalcification it is frozen and then thawed They found that this procedure shortened the clotting time of citrated recalcified plasma from 3–6 minutes to 1½–½ minutes One can also destroy the platelets by the addition of 3 volumes of distilled water to one volume of citrated plasma Iso osmicity is then re-established by adding one volume of a 3 6 per cent solution of sodium chloride We have studied the effect of the destruction of platelets on the Howell's time of citrated platelet rich plasma from the patient's blood

Table 4 shows that there is a considerable shortening of the clotting time after destruction of the platelets However this clotting time is still abnormally prolonged

Control experiments on platelet free plasma showed no effect of freezing on the clotting time However the addition of distilled water to the platelet free plasma resulted in a shortening of the clotting time This effect may be due to dilution of the anticoagulant by the distilled water since the addition of saline instead of distilled water produced a similar effect

c Effect of dilution on Howell's time

present in the patient's plasma. Both substances are anticlotting but not antithrombic. They both are thermostable. It became necessary to investigate whether the patient's plasma exhibited enhanced antiproteolytic or antifibrinolytic activity.

TABLE 9.—*Effect of Incubation of Patient's Plasma with Thrombin. Thrombin Added to Defibrinated Plasma, the Mixtures Incubated 15 Minutes at 37 C. and the Clotting Time on Fibrinogen Measured at 37 C. Defibrination Was Done by Heating at 56 C. for 5 Minutes*

Reagent and thrombin	Normal plasma	Patient plasma	Saline	Fibrinogen Solution	Clotting time
					seconds
0.4	0	0	0.1	0.5	9
0.4	0.1	0	0	0.5	36
0.4	0	0.1	0	0.5	4

TABLE 10

A. Fibrinolysin 1 cc. + Citrated Plasma 0.05 cc. + Thrombin 0.1 cc. Reading Done after Incubation at 37 C. for 18 Hours

Dilution of fibrinolysin	Lysis	
	Patient plasma	Normal plasma
1/1	+	+
1/2	+	0
1/4	0	0
1/8	0	0
1/16	0	0
1/32	0	0
1/64	0	0

B. Fibrinolysin 0.2 cc. Plasma 0.2 cc. Diluted with 0.2 per cent Solution of Fibrinogen. Reading Done after Incubation at 37 C. for 18 Hours

Dilution of plasma	Lysis	
	Patient plasma	Normal Plasma
1/1	0	0
1/2	0	0
1/4	0	0
1/8	+	0
1/16	+	+
1/32	+	+
1/64	+	+
1/128	+	+
1/256	+	+

As a source of fibrinolysin we used a preparation of chloroform plasma (Nolf Tagnon). The fibrinolysin preparation was added to citrated plasma and a clot was obtained by the addition of thrombin. The disappearance of the clot was taken as a measure of the intensity of the fibrinolysis.

8 Effect of thrombin on citrated plasma absence of immediate antithrombin

The preparation used was obtained from the Roussel s laboratories as a dry powder 50 mg of which assayed 12 Iowa units Fifty milligrams was dissolved in 2 cc of physiologic saline At all dilutions used (table 8) it was impossible to detect a significant immediate antithrombic action in the patient s plasma

TABLE 6 —*Effect of Normal Plasma Fraction I from Normal Plasma and Fraction I from Hemophilic Plasma on Clotting Time of Platelet s Plasma Reagents in cc*

Patient s plasma	Normal plasma	Fraction I normal	Fraction I hemophilia	Clotting Time
				minutes
0 1	1	0	0	40
0 1	0	1	0	120
0 1	0	0	1	120

* *After addition of 1 1 cc of CaCl to each tube at 37 C*

TABLE 7 —*Effect of Dilution of Thromboplastin on Prothrombin Time (0 1 cc Citrated Plasma + 0 1 cc Solution of Thromboplastin + 0 1 cc CaCl 0 025 N)*

Dilution of thromboplastin	Prothrombin time (seconds) of	
	Patient s plasma	Normal plasma
1/1	20	18
1/10	32	26
1/50	63	50
1/100	84	62
1/200	127	83
1/500	180	116
1/1000	390	140

TABLE 8 —*Effect of Thrombin on Citrated Plasma (10 5 cc Plasma + 0 5 of Thrombin Solution at 37 C)*

Dilution of thrombin	Clotting time of normal human citrated plasma	Clotting time of patient s citrated plasma
	seconds	*seconds*
1/1	11 5	11
1/2	19 5	20
1/4	36	37
1/16	100	106
1/32	228	270

9 Progressive antithrombin

Normal serum or plasma contain an antithrombin which is called progressive because it inactivates thrombin when incubated with thrombin Table 9 shows that the patient s plasma did not inactivate thrombin more actively than did normal plasma and did not therefore contain more progressive antithrombin than did normal plasma

10 Antifibrinolytic activity of the plasma

It was decided to test this activity in the patient s plasma because there are similarities between the trypsin inhibitor from soya bean and the anticoagulant

principle acts on the first phase of the coagulation phenomenon since we have shown that it is not antithrombin It would appear therefore that it opposes the transformation of prothrombin into thrombin

The anticoagulant could conceivably oppose this transformation by one of three possible mechanisms (1) by an action on calcium that this was not the case is affected by the fact that the blood calcium was normal and that varying the amount of calcium did not correct the prolonged clotting time (2) by an action on prothrombin this seems unlikely since the prothrombin time was normal on the other hand we have mixed 0 1 cc of the patient s plasma with 1 cc of prothrombin poor plasma (obtained from a patient treated with dicumarol) containing 33 per cent of the normal level of prothrombin The clotting time of the mixture (38-48 minutes) was found to be similar to that of a control (38-45 minutes) (3) by an action on thromboplastin this is rendered unlikely by the fact that small quantities of thromboplastin added to the patient s plasma corrected the clotting defect quite completely The antithromboplastin described by Tocantins [6] in contrast to the anticoagulant described here, is destroyed by heating at 50 C

Other hypothes s should be briefly considered According to Lenggenhager [7] the thromboplastin originating from platelets exists as a prothromboplastin and is activated by contact with foreign surfaces and calcium while the thromboplastin derived from cellular material exists in an active form According to this hypothesis the anticoagulant described here could conceivably work by opposing the activation of the prothromboplastin

According to Nolf [8] Howell [9] Patek and Stetson [10] Patek and Taylor [11] Feissly [1] Bendien and Van Creveld [12] blood plasma contains an essential factor of blood coagulation distinct from prothrombin This factor exists in plasma as an inactive precursor It is thermolabile and is bound to the englobulin fraction of plasma It is call-d antihemophilic factor globulin substance plasma thromboplastin etc

According to Frederica [14] and independently to Ferguson [13] the plasma tryptase is probably the active principle which in association with calcium and the platelets activates prothrombin This author thinks that component A of prothrombin described by Quick [1] is identical with the plasma tryptase

A definite explanation of this mechanism of action of the anticoagulant described here must await a better understanding of the mechanism of normal coagulation itself Our impression is that this anticoagulant acts on either the mechanism of activation of prothromboplastin or the antihemophilic substance or the plasma tryptase

An interesting question is whether our anticoagulant is identical with the anti clotting substances described in 3 different publications [15] [16] [4] Certain common features suggest an identity these are the thermostability lack of antithrombin action lack of action on the prothrombin time

The substance described by Munro[4] [17] appears to be a γ globulin

Summary

A new observation of a hemorrhagic diathesis associated with the presence of an anticoagulant in the circulating blood is reported here The patient was a 21

11 Finally we have observed that the quantity of anticoagulant agent in the patient s plasma increased from August to November 1946 This is shown by the fact that in August a mixture of 1 volume of citrated normal plasma and 1 volume of citrated patient s plasma after addition of calcium clotted in 50 to 60 minutes while in November in order to obtain the same clotting time it was necessary to add 4 volumes of normal plasma to one volume of the patient s plasma It is difficult to say at the present whether this increase was the result of the transfusion of blood as was the case in the patient described by Munro [17]

Discussion

The patient discussed in this communication was clinically a hemophiliac This *diagnosis was based on a prolonged clotting time a normal bleeding time* and the occurrence of hemorrhagic episodes and joint hemorrhages The disease started at the age of 18 months However certain aspects of the case were not in agreement with the diagnosis of hemophilia among which the absence of a family history of the disease and the lack of effect of blood transfusions Also atypical were the lack of effect of the addition of either normal plasma or fraction I of Cohn on the clotting time in vitro of the patient s blood Finally and contrary to what occurs in true hemophilia the blood or plasma from the patient had a definite anticlotting effect on normal blood or plasma

There is little doubt that the blood of this patient contained a circulating *anti* coagulant Munro [4, 5] has reported a similar occurrence in a patient classified as hemophilic In Munro s case the appearance of the anticoagulant followed the use of repeated blood transfusions In the case reported here su h an etiology seems unlikely in view of the fact that the patient had received one single transfusion before the time that the circulating anticoagulant was observed in his blood This single transfusion was given him when he was a child Later transfusions given after the circulating coagulant was observed had no effect on the clotting time or if they had any effect it was in the direction of *prolonging* the clotting time al though this prolonging effect was difficult to demonstrate since the blood before transfusion clotted in the very long time of 6 hours at 37 C We have given this patient one transfusion only because of the detrimental effect of this treatment in this typ of cases as described by Munro [17]

The anticoagulant present in the plasma of this patient is thermostable We were able to heat the plasma at 65 C for 30 minute without causing the disap pearance of the anticlotting activity At ice box temperature the activity is main tained for 8 days at least At room temperature the activity decreases after 48 hours The anticoagulant appears not to be identical with heparin since we have observed *no neutralizing effect from the addition of toluidine* blue Furthermore quantities of protamine between 0 001 mg and 0 1 mg for 1 cc of plasma did not affect the clotting time

The anticoagulant was not an antithrombin and did not appear to be antifibrin olytic The prothrombin time determined by the method of Quick was not sig nificantly altered

Concerning the mechanism of action of the anticoagulant one can say that this

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STUDIES ON AN UNDETERMINED CIRCULATING ANTICOAGULANT CASE REPORT AND LABORATORY FINDINGS

By D G Dieter M D M Spooner M A F J Pohle M D †

Introduction

IN 1940 Lozner Joliffe and Taylor[1] reported the case of a 61 year old male Negro with an undetermined circulating anticoagulant More recently Lawrence and Johnson and Munro[3] have reported studies on male patients previously diagnosed as hemophiliacs who developed a circulating anticoagulant following numerous blood transfusions Madison and Quick[4] presented a case and reviewed several other cases of female patients with hemorrhagic diatheses characterized by prolonged coagulation times

The circulating anticoagulants present in the patients of Lozner et al [1] Lawrence and Johnson and Munro [3] although never identified had the following common characteristics (1) they prolonged the coagulation time of normal blood (2) they were thermostable (3) they showed no antithrombic activity (4) they were not neutralized by protamine (5) they did not pass through semipermeable membranes (6) they were not extracted by ether Lozner and his associates found that the anticoagulant material which they described was not associated with the euglobulin fraction which contained the antihemophilic property of plasma Munro found that the anticoagulant with which he was working was not precipitated as euglobulin Munro noted that the anticoagulant when precipitated as a globulin maintained an anticoagulant activity equal to that of the plasma from which it was derived It was also stable in a pH of 6 5 to 11 0 So far as history and clinical observation is concerned it is relatively certain that the patient described by Lozner and Taylor was not a case of hemophilia

The patients reported by Madison and Quick[4] and referred to as hemophilia like were probably similar to those already described although the data presented are insufficient to make a positive statement The fact that the patients discussed by these authors in spite of the fact that they may have had different diseases had an increased coagulation time as the only abnormal finding suggests an anticoagulant with the same characteristics as those investigated by Lozner et al [1] Lawrence and Johnson [2] and Munro[3]

This paper presents the history hematologic studies and clinical course of a patient with a prolonged coagulation time due to an undetermined anticoagulant This anticoagulant appears to be similar to those which were present in the above mentioned cases

Report of Case

The patient a 68 year old white male banker was admitted to Madison General Hospital on October 12 1946 compl ining of lo of appetite for five days prior to admission This as followed by generalized muscul aches pains in the extreme lower abdomen and gross hematuria

From the Departments of Medicine and Physiology University of W sconsin Medical School Madison Wisco n

Th s study was a ded by a gra t f om the Wisconsin Al mni Research Foundation

† Deceased

year old male appearing by clinical evaluation to have hemophilia but without a family history of hemophilia The blood and plasma were strongly anticlotting and had a very long clotting time The clotting time of recalcified citrated plasma was greatly delayed by removing the platelets Freezing and thawing of platelet rich plasma resulted in a marked shortening of the clotting time Dilution of the plasma shortened the clotting time while the addition of calcium and storage of the plasma had no effect

The prolonged clotting time was not corrected by the addition of normal plasma or plasma fractions having antihemophilic activity The prothrombin time was nearly normal Small quantities of thromboplastin were very effective in shortening the clotting time The anticoagulant had no antithrombin activity The progressive antithrombin and antifibrinolysin of the patient s plasma were normal

The anticoagulant acts during the first phase of coagulation by inhibiting an (plasma) activator of prothrombin It appears to be identical with the anticoagulant described in three previous publications from the United States

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This paper presents the history hematologic studies and clinical course of a patient with a prolonged coagulation time due to an undetermined anticoagulant This anticoagulant appears to be similar to those which were present in the above mentioned cases

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The patient a 68 year old white male banker was admitted to Madison General Hospital on October 12 1946 compla n ng of loss of appetite for five days prior to admission This was followed by generalized muscular aches p ns in the extreme lower abdomen and gro s hematuria

From the Departments of Medicine and Physiology University of Wisconsin Medical School Madison Wisconsin

This study was aided by a grant from the Wisconsin Alumni Research Foundation

† Deceased

History of present illness The patient had apparently been in good health until eight months before admission at which time he developed what appeared to be a contact dermatitis on the back of his right hand which gradually spread over both arms and back This was treated with sulfathiazol ointment with no improvement Two weeks after the onset he was admitted to the State of Wisconsin General Hospital where he was treated with boric acid compresses and boric ointment He recovered and was discharged on the sixth hospital day

The patient was readmitted to the Wisconsin General Hospital one month later because of recurrence of the skin lesions The condition was diagnosed at this time as pemphigus and the patient was treated with stovarsal 50 mg before breakfast for three days After a three day interval in which no drug was given the dose of stovarsal was increased to 100 mg daily With this regimen the process slowly subsided and the dose was gradually increased to 250 mg of stovarsal daily for three successive days followed by three day rest periods The patient was discharged on the sixty seventh hospital day and followed as an out patient for the next three months While an out patient he received the same therapy for two months with complete disappearance of all skin lesions except for a small bullous lesion on the left hand

Approximately two months prior to admission to Madison General Hospital the patient developed an acute painful swelling of the left elbow This was thought to be an acute bursitis The joint was aspirated and blood was obtained A pressure bandage was applied and no further bleeding occurred Later 2 large ecchymotic areas developed on his right arm while he was bait casting These gradually subsided

Past Medical History The patient had a benign bladder tumor fulgurated twelve years prior to admission

Family History No other members of the patient s family had had any hemorrhagic diatheses

Physical Examination The patient was a well developed fairly well nourished man who did not appear acutely ill The temperature was 99 6 F the pulse rate was 80 the blood pressure 150/80 mm Hg

The vessels of the optic fundi showed a grade 2 sclerosis with no evidence of hemorrhage or exudates A few palpable cervical and axillary nodes were present There were a few scattered fine crepitant rales in the posterior lung fields bilaterally The left cardiac border was percussed 1 cm outside the mid clavicular line There were grade 1 apical and aortic systolic murmurs which were not transmitted The liver edge was palapable 2 cm below the right costal margin and was nontender The spleen was not palpable There was moderate tenderness over the left side of the abdomen at the level of the umbilicus Bilateral indirect inguinal hernias were present Vibratory sense was absent in the left leg Sternal tenderness was noted

The preliminary laboratory studies were as shown in table 1

On systoscopic examination blood was seen coming from the left ureter

X ray studies of the chest were negative for signs of tuberculosis and pneumonitis A retrograde pyelogram of the left kidney revealed that the kidney was displaced upward X ray studies of the kidneys ureter and bladder taken after the retrograde pyelogram showed small areas of opaque material in the region of the left kidney pelvis which was interpreted as the contrast material of the retrograde pyelogram incorporated in blood clots

Clinical course The patient s stay in the hospital was characterized by exacerbations of bleeding followed by a quiescent period during which the patient improved On one occasion his condition appeared terminal Auricular fibrillation developed with evidence of decompensation The blood nonprotein nitrogen became elevated and symptoms and signs of uremia followed There was a gradual recovery from this acute phase Numerous episodes of spontaneous hemorrhages occurred which involved the upper and lower extremities At one time the hemorrhage into the left arm was so extensive that a left radial palsy resulted There were intracapsular hemorrhages into the shoulder elbow hip and knee joints On two occasions bleeding occurred into the tongue with extension into the sublingual region and the pharynx There were two episodes of gross hematuria On several occasions the physical findings were compatible with intra abdominal and retroperitoneal bleeding There was one episode of severe low back pain associated with clonic contractions of the muscles of the back and both lower extremities It was thought that this resulted from an extensive hematoma compressing the spinal cord At one time there was hemorrhage into both parotid capsules Progressively however it appeared that the patient was slowly improving for the episodes of bleeding were not as frequent and the hemorrhages

were less extensive Because of the futility of the treatment and the low morale of the patient he was discharged on August 16 194 His condition has improved subjectively since discharge There has been increased appetite The radial nerve palsy has disappeared There have been no new episodes of bleeding up to the present time even though a coagulation time done after two months at home was 90 minutes

Therapy Along with the supportive measures that were necessary to control pain combat the persistent anemia and maintain the patient in the best possible state of nutrition and hydration specific therapeutic agents were employed in an attempt to control the bleeding tendency Blood transfusions plasma concentrated albumin intravenous calcium gluconate hemostatic serum vitamins C K P and glucoside of quercetin (rutin) were administered until the condition could b further investigated When it was disco ered by adding the patient s plasma to normal whole blood that the coagulation defect was due to a circulating anticoagulant which prolonged coagulation time of normal blood 50 cc of 1 per cent solution of salmine protamine were gi en intravenously daily for fourteen days [7, 8] This therapy failed to affect the coagulation time materially

TABLE 1 —*Resume of Laboratory Findings*

Erythrocyte count	1 570 000 to 4 130 000 cells per cu mm blood
Hemoglobin	5 to 12 Gm per 100 cc blood
Leukocyte count	8 200 to 19 500 cells per cu mm blood
Differential	neutrophiles increased to 87% during febrile state
Sedimentation rate (Wintrobe)	45 mm in one hour
Blood non protein nitrogen	29—5 mg per 100 cc
Hanger s test (cephalin cholesterol flocculation)	2+ in 48 hours
Fasting blood sugar	87 to 129 mg per 100 cc
Icterus index	8 to 50 units
Total serum protein	4 5 to 6 4 Gm per 100 cc
albumin	1 5 to 3 1 Gm per 100 cc
globulin	1 8 to 4 4 Gm per 100 cc
Serology	negati e

METHODS

The experimental technics used in these investigations were kept as uniform as possible All blood samples were drawn in cooled oiled syringes When citrated plasma was used the blood was obtained by venipuncture and mixed with 3 8 per cent sodium citrate in the ratio of 9 1 The mixture was kept in an ice bath until used With the exception of the studies on the effect of high and low centrifuging and platelet activity the blood was centrifuged at 1500 rpm for ten minutes at 4 C When uncitrated plasma was used lusteroid tubes were substituted for glass

The Lee White method was used in determining the coagulation times of whole blood In studying the anticoagulant glass tubes 13 mm in diameter were placed in a water bath at 37 C and all clotting times were determined at that temperature The reagents used in all experiments were also kept at 37 C The volumes utilized in the individual studies were maintained at 1 ml except in several specified instances With a calcium chloride concentration of 0 025 M the coagulation time of recalcified normal plasma was 2–3 minutes that of normal uncitrated plasma 4 5 to 5 5 minutes

Effect of citrated normal human plasma on the coagulation time of the patient's blood To determine whether human plasma in minute quantities would shorten the coagulation time of the patient's blood the proportions were set up as shown in table 3 Small amounts of normal plasma shortened the coagulation time of the patient's blood to some extent, but did not return the coagulation time to normal limits The coagulation time changed very little when larger amounts of normal plasma were added to the patient's blood

Effect of patient's citrated plasma on the coagulation time of normal blood Amounts of patient's plasma varying from 0.1 ml to 0.003 ml were added to 2 ml of normal blood In all cases, isotonic salt solution was added in sufficient quantity to make the volume of the patient's blood preparation equal to 0.1 ml The results are given

TABLE 2 —*Special Hematologic Studies*

Platelet count (direct wet method)	216 000 to 324 000 per cu mm blood
Bleeding time (Duke)	1.5 to 3.5 minutes
Coagulation time (capillary tube)	4.5 to 45 minutes
(Lee White)	90 to 270 minutes
Clot retraction	normal in 24 hours at 37° C
Tourniquet test (Rumpel Leede)	normal
Prothrombin concentration (Quick)	91 to 105%
Fibrinogen	normal
Ascorbic acid (fasting whole blood)	0.40 mg per 100 cc
(fasting plasma)	0.13 mg per 100 cc
Antithrombin activity of serum (Wilson)	normal

TABLE 3 —*Effect of Citrated Normal Human Plasma on the Coagulation Time of the Patient's Blood*

	Coagulation Time
	min
2.0 ml patient's blood (control)	127
2.0 ml patient's blood +0.01 ml normal plasma	84
2.0 ml patient's blood +0.03 ml normal plasma	83
2.0 ml patient's blood +0.05 ml normal plasma	68
2.0 ml patient's blood +0.10 ml normal plasma	129
2.0 ml patient's blood +0.20 ml normal plasma	120

in table 4 These data show that an anticoagulant activity was present in the patient's plasma

Effect of the patient's uncitrated plasma on normal uncitrated plasma It was found that when dilutions ranging from 0.05 to 0.20 ml of the patient's uncitrated plasma were added to 0.4 ml of normal uncitrated plasma with 0.75 M sodium chloride added to make a volume of 1.0 ml the effect although not as marked as that shown in Tables 4 and 5 showed some tendency toward prolongation

Effect of patient's citrated plasma on normal citrated plasma The above experiment was repeated with citrated normal and patient's plasma to which was added 0.4 ml of 0.025 M calcium chloride and 0.15 M sodium chloride to make a total of 1.0 ml The results of this and a control are shown in table 6

The data show that when increased amounts of the patient's plasma previously recalcified are added to 0.4 ml of normal plasma the coagulation time is increased. The effect is not extremely marked until 0.4 cc of the patient's plasma is added in which case it is shown that equal amounts of normal plasma and patient's

TABLE 4.—*Effect of Patient's Citrated Plasma on the Coagulation Time of Normal Blood*

	Coagulation Time
	min
2.0 ml normal blood (control)	12
2.0 ml normal blood +0.003 ml patient's plasma	18
2.0 ml normal blood +0.005 ml patient's plasma	24
2.0 ml normal blood +0.010 ml patient's plasma	25
2.0 ml normal blood +0.030 ml patient's plasma	45
2.0 ml normal blood +0.050 ml patient's plasma	58
2.0 ml normal blood +0.100 ml patient's plasma	82

TABLE 5.—*Effect of Patient's Uncitrated Plasma on Normal Uncitrated Plasma*

Patient's Plasma	Normal Plasma	0.15 M NaCl	Coagulation Time
ml	*ml*	*ml*	*c*
0.00	0.40	0.60	4.5
0.05	0.40	0.55	7.0
0.15	0.40	0.45	13.0
0.20	0.40	0.40	7.0
0.20	0.00	0.80	149.0
0.00	0.20	0.80	4.5

TABLE 6.—*Effect of Patient's Citrated Plasma on Normal Citrated Plasma. Mixture of material added to 0.4 ml of normal citrated plasma and recalcified with 0.4 ml of 0.025 M calcium chloride*

Normal Plasma	Patient's Plasma	Control Plasma	0.15 M NaCl	Coagulation Time
ml	*ml*	*ml*	*ml*	*cc*
0.40	0.00	0.00	0.40	2.5
0.40	0.05	0.00	0.35	5.5
0.40	0.15	0.00	0.25	6.5
0.40	0.20	0.00	0. 0	8.0
0.40	0.40	0.00	0.00	23.0
0.40	0.00	0.00	0.40	2.5
0.40	0.00	0.05	0.35	3.0
0.40	0.00	0.15	0.25	2.5
0.40	0.00	0.20	0.20	2.5
0.40	0.00	0.40	0.00	3.0

plasma give a coagulation time of over 23 minutes. A control experiment adding recalcified normal plasma to normal plasma did not show this type of change.

Effect of high and low centrifuging on patient's citrated and uncitrated plasma as compared with the normal. To rule out hemophilia further, samples of citrated and un

citrated patient s and normal blood were submitted to high (3000 rpm for five minutes) and low (1000 rpm for five minutes) centrifuging Quick[4 5] has observed that after high centrifuging the coagulation time of recalcified hemophilic plasma is considerably slower than that obtained by spontaneous sedimentation or low centrifugation This could not be demonstrated on the plasma of this patient The findings are recorded in table 7

Samples of citrated and uncitrated blood from the patient and from a normal individual were submitted to centrifugation at 3000 rpm per minute for five minutes and also at 1000 rpm for five minutes This experiment was done in order to determine whether or not the same relation to spinning blood at 3000 rpm and 1000 rpm which Quick found in hemophilia applied to the blood of this patient No such similarity was obtained

Effect of salmine protamine and toluidine blue on the coagulation times of patient s and normal blood[6–8] Although it has been stated above that salmine protamine was

TABLE 7 —*Effect of High and Low Centrifuging on Patient s Citrated and Uncitrated Plasma as Compared with the Normal*

	Normal Plasma	Patient s Plasma	0 15 M NaCl	0 025 M CaCl	Coagulation Time
	ml	*ml*	*ml*	*ml*	*m*
High speed centrifuging of uncitrated plasma	0 00	0 20	0 80	0 00	68 0
	0 20	0 00	0 80	0 00	4 0
Low speed centrifuging of uncitrated plasma	0 00	0 20	0 80	0 00	149 0
	0 20	0 00	0 80	0 00	4 5
High speed centrifuging of recalcified plasma	0 00	0 20	0 60	0 40	9 5
	0 20	0 00	0 60	0 40	2 0
Low speed centrifuging of recalcified plasma	0 00	0 20	0 60	0 40	11 0
	0 20	0 00	0 60	0 40	3 0

It was found that by employing the technic outlined in the explanation of methods normal blood remained unclotted for over 90 minutes and the patient s blood was not coagulated 6 hours later

used intravenously with no appreciable reduction of the clotting time experiments were performed to test the effectiveness of it and toluidine blue in vitro Amounts of a 0 1 per cent solution of the two drugs ranging from 0 02 to 0 10 ml were added to 0 20 ml of the patient s citrated plasma which was diluted to 1 0 ml with 0 15 M sodium chloride and recalcified with 0 40 ml of 0 025 M calcium chloride It was found that these drugs further prolonged the coagulation time of recalcified patient s plasma This experiment was repeated using normal blood with similar results

To test the effectiveness of these drugs to neutralize the anticoagulant properties of heparin 1 unit of heparin was added to 0 20 ml of normal plasma The reagents salmine protamine toluidine blue 0 15 M sodium chloride and 0 025 M calcium chloride were added in the same order and amounts as discussed in the previous paragraph These studies showed that 1 unit of heparin prolonged the coagulation time of normal recalcified plasma to 11 minutes (normal 2–3 minutes) and that

0 02 ml of either of the two drugs being tested reduced the clotting time to 4 5 minutes Amounts in excess of 0 02 ml of salmine protamine and toluidine blue prolonged the coagulation times

Studies on Platelet Fragility[9]

Studies on platelet fragility were performed according to the method of Muhre Bogart and Hogan[9] by combining the patient s recalcified plasma with concentrations of sodium chloride varying from 0 33 to 2 5 per cent The results of these experiments show that the clotting time obtained with 0 8 per cent saline solution was found to be the same as that obtained with recalcified plasma i e 10 minutes

TABLE 8 —*Studies on Platelet Activity*

Recalcification was carried out in each instance with 0 40 ml of 0 025 M $CaCl_2$

Pl t let Poo Pl sm	Sal n S spen on f Plat l ts	0 15 M N Cl	Co gulat on T m
ml	*ml*	*ml*	*m*
0 20 normal	0 20 normal	0 20	2 0
0 20 normal	0 20 patient	0 20	3 0
0 20 normal	0 00 —	0 40	5 5
0 20 patient	0 00 —	0 40	16 0
0 20 patient	0 20 patient	0 20	12 0
0 20 patient	0 20 normal	0 20	10 0

TABLE 9

Test System 0 20 ml patient s citrated plasma +0 40 ml normal plasma +0 40 ml 0 15 M NaCl + 0 40 ml 0 025 M $CaCl_2$

C d ti t wh h P t t s Pl sm w S bj t d	Coagul tio Time
	m n te
4 degrees C for 24 hours	3 0
Room temperature for 4 hours	3 0
61 degrees C for 10 minutes	7 5
Unheated plasma	6 5

When the sodium chloride solution became hypertonic the coagulation time was prolonged This procedure was repeated on normal plasma with similar findings

Studies on Platelet Activity

To rule out further the possibility that the platelets were responsible for the coagulation defect a study of platelet activity was carried out according to the method described by Patek and Stetson[11] The technic for drawing and citrating the blood was that of previous experiments except that paraffin coated tubes were used instead of glass The pipets used in these studies were coated in the inside with a thin film of collodion Plasma was obtained by centrifuging the blood at 1500 rpm for ten minutes This plasma was withdrawn and centrifuged at 4200 rpm for fifteen minutes to produce platelet poor plasma The platelets were separated

from the platelet poor plasma and were washed in normal saline and resuspended in a volume of 0 15 M sodium chloride equal to the original volume of plasma The results of this experiment are shown in table 8

The data indicate that there was no essential difference in the activity of platelet suspension obtained by this technic and that obtained from normal blood and from the blood of the patient

Effect of cold storage room temperature and heat on coagulation time of patient s citrated plasma Citrated samples of the patient s plasma were subjected to 4 degrees Centigrade for twenty four hours room temperature for three to four hours and 61 degrees Centigrade for ten minutes It was found that heat did not destroy the anticoagulant but when the plasma was allowed to stand at room temperature for four hours or in a refrigerator at 4 degrees Centigrade for twenty four hours the coagulation time of patient s plasma was normal (2–3 minutes) and the anti coagulant action on normal plasma had disappeared as shown in table 9

Effect of Dialysis

Ten ml of the patient s plasma prepared in the usual manner were placed in a viscose casing bag and allowed to rotate in distilled water for a period of twenty four hours at 4 degrees centigrade The dialysate and the contents of the bag were found to have no anticoagulant activity

Electrophoretic Analysis

The a/g ratio was found to be 0 63 The patient s plasma was submitted to electrophoretic analysis Increased amounts of each of the globulin fractions were reported However as a whole the pattern of the globulin proteins was not re markable The actual analyses for albumin and globulin based on the Shirring diagram was 2 9 Gm per cent albumin 4 6 Gm per cent globulin per 100 ml of plasma

Discussion

The known hemorrhagic diathases such as increased capillary fragility thrombocytopenic purpura hemorrhagica afibrinogenemia hemophilia pseudohemophilia hypoprothrombinemia athromboplastinopenia and afibrinogenopenia have been ruled out either by the history or laboratory findings as summarized in table 1 or by the therapy that the patient received

The discovery that the patient s plasma prolonged the coagulation time of normal blood established the presence of a circulating anticoagulant The investigative results of the patient s coagulation defect do not place this anticoagulant in four of the five categories postulated by Quick [5] namely decalcifying agents antiprothrombins antithrombins and fibrinogen antagonists The question of whether antithromboplastin is present is not clearly answered

The presence of any one of these factors as the cause for the defect in the coagulation mechanism although not positively ruled out has at least been eliminated except for antithromboplastin The studies show that the patient s anticoagulant was not destroyed at 61 C for ten minutes while the antithromboplastin described

by Tocantins was destroyed by heat Munro s[3] argument that there possibly exists more than one antithromboplastin is logical

The investigative work conducted on this patient was as complete as possible with the available technics The data accumulated are similar to those obtained by Lozner et al [1] Lawrence and Johnson [2] and Munro[3] regarding the nature of the anticoagulant with one probable exception namely their anticoagulant was stable to storage as well as heat a finding which is probably not altogether true for this patient so far as one may judge by a comparison of table 3 and 8 However if one can compare the data of whole blood and plasma as is necessary in these experiments one would judge that at least part of the antithromboplastic or anti coagulant activity was destroyed Neither the anticoagulant described by these workers nor the one described here passed through semipermeable membranes It is thought however that the results obtained on these studies are comparable to those reported by other investigators on patients with similar afflictions and hence this case probably belongs in the same or a similar category

The factor which precipitated this hemorrhagic diathesis is as obscure as in the case reported by Lozner et al [1] It is possible that the pemphigus was the exciting factor Stovarsol cannot be eliminated even though routine laboratory studies during the period that the drug was used were in no way unusual Coagulation times (capillary tube method) of 5 patients with pemphigus who received stovarsol therapy were within normal limits Another possibility is that the prolonged coagulation time would have occurred spontaneously Unlike the patients of Lawrence and Johnson and Munro [3] our patient had a coagulation defect prior to the administration of multiple transfusions

Conclusions

The results of various studies upon a patient with a coagulation defect are re ported The cause of this defect was found to be a circulating anticoagulant whose exact nature remains obscure

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THE RELATIONSHIP BETWEEN HEPARIN DOSAGE AND CLOTTING TIME

By L B JAQUES M A PH D , AND ANN G RICKER M A

THE GREAT interest of Dr Minot in all branches of hematology has always included an appreciation of the problems involving the anticoagulants The recent development of anticoagulant therapy has increased this interest We therefore are pleased to present as an indication of our esteem a recent study of factors affecting the action of heparin in vivo We believe that a study of this nature is of value in leading to an understanding of the various physiologic factors involved in this action and hence to an appreciation of the principles involved in its administration

Heparin when used experimentally or clinically is usually controlled by the determination of the prolonged clotting time produced It was shown in a previous paper[1] that the value of the clotting time produced will depend on both the coagulant power of the blood and the ability of the body to remove heparin from the circulation Crafoord reported that the blood became more resistant to the action of heparin after operation suggesting an increase in coagulability Following this lead De Takats[3] Waugh and Ruddick[4] and Whittaker[5] have suggested tests of blood coagulability based on the resistance of the blood to the anticoagulant power of heparin In a continuation of the previous study further observations of the effect of heparin dosage on the clotting time in the dog have been made These observations give a clearer concept of the factors involved both in the use of heparin as a test of the clotting function of the blood and also in its administration

METHODS

Dogs of 10 to 20 Kg body weight were used The experiments were conducted on trained unanesthetized animals unless otherwise indicated Unless otherwise stated blood samples were taken by venopuncture from superficial veins through the skin Care was taken to ensure that the sample was taken expeditiously without trauma The syringe and needle were first rinsed with saline and emptied of air saline being left in the needle Normally 2 cc of blood were taken and the first 0 5 cc and last 0 5 cc of blood were discarded If larger quantities of blood were required the same technic was used larger amounts of the first and last of the sample being discarded Clotting times were determined at 37 C in the coagulometer described by Murray Jaques Perrett and Best[6]

We are indebted to the Connaught Medical Research Laboratories University of Toronto for a generous supply of heparin This was received as a solution of 1000 Connaught units/cc (9 5 mg per cc) of the sodium salt of beef heparin The unit is the anticoagulant activity of 1/100 mg of the crystalline barium salt as prepared by the method of Charles and Scott[7] This unit is as closely as one can ascertain the same as the provisional International Unit[8] which is expected to replace it and also the original unit used by Howell In these studies the heparin was injected intravenously

CLOTTING TIME RESPONSE TO HEPARIN IN VITRO

When the clotting time is determined with varying amounts of heparin added to the blood in vitro a curve such as that of *a* in figure 1 is obtained The practical

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was drawn to give the best fit for all the points without necessarily passing through the normal value Typical results obtained in a series of 15 dogs are shown in table 1 The clotting times as those in figure 1 were determined on blood removed from an exposed femoral vein It can be seen that the range for the heparin sensitivity values for the animals was from 0 50 to 48 8 These extreme values were for only two animals representing the hyperreactors and hyporeactors of De Takats The values for 50 per cent of the animals were in the range 2 3 to 3 2 while 5 (31 per cent) were moderate hyperreactors giving values of 3 8 to 7 6 The difference between different animals with regard to their sensitivity to heparin is a relatively permanent difference Determinations on the same animal, 10 days apart shown in table 2 give sensitivity values agreeing to within 10 per cent while the

TABLE 1 —*Sensitivity of the Blood to the Action of Heparin in Dogs*

Dog	Normal Cl T† Min	Heparin sensitivity Value	Cl T with heparin	
			0 1 units / Min	0 5 units / Min
A 19	3 6	24 2	α	α
A 20	2 0	50	2 2	3 6
A 21	2 0	48 8	α	α
A 22	1 0	5 48	3 6	α
A 26	4 0	4 12	10 5	α
A 31	1 0	3 22	2 1	41
A 34	2 5	2 80	4 7	63
A 35	2 0	7 28	10 4	α
A 36	3 0	2 84	5 8	78
A 3	1 1	4 30	4 7	α
A 38	2 9	2 31	5 0	42
A 39	3 7	2 65	8 7	α
A 42	0 9	2 38	1 7	15
A 43	1 0	2 76	2 0	25
A 44	2 0	2 46	3 6	34
Median	2 0	2 8	4 7	78

Blood samples taken from exposed vein
† Cl T —Clotting Time α—>90

difference between the average normal dog and the hyperreactors is one of several hundred per cent

Another method of measuring sensitivity to heparin although by no means as informative is to report the clotting time for a single dose of heparin This has been done in table 1 for 0 1 and 0 5 units of heparin/cc Also reported is the normal or control clotting time i e the clotting time without added heparin The value of the clotting time for 0 1 units of heparin was from 1 7 minutes (almost the same as the normal) to over 24 hours The median value was 4 7 minutes Another way of giving the values is the concentration of heparin required to double the normal clotting time This was 0 008 units for dog A 21 and 0 6 units for dog A 20 the median value for the series was 0 11 units There appeared to be no correlation be

significance of this curve is that a *certain level of heparin is required before there* is any significant effect on clotting time whereas increases in heparin concentration beyond this value result in the clotting time being very markedly increased *for slight increases in heparin concentration* Several empirical equations have been suggested for this curve [1, 9, 10] However a simple procedure is to plot the data on semilogarithmic paper whereupon a straight line is obtained In figure 1 this has been done to give curve *b* The same procedure has previously been recommended for the relation between thrombin concentration and clotting time[11] and appears to be a consequence of the determination of clotting times since a similar

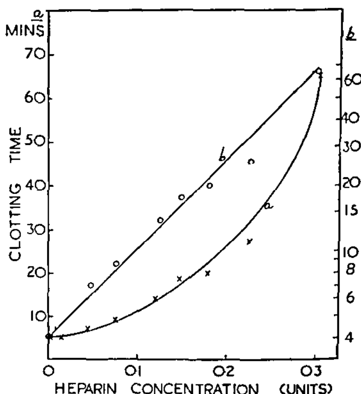


Fig 1 Effect of heparin concentration in vitro on clotting time Blood samples taken from the exposed femoral vein and 1 cc added to the quantities of heparin shown

logarithmic relation can be obtained for many coagulant and anticoagulant substances

It would appear of practical importance to have some single value to express the sensitivity of a blood sample to the anticoagulant action of heparin This is easily achieved by taking the slope of the straight line obtained on semilog paper This gives us the *heparin sensitivity value* and is calculated from $\frac{\log T_2 - \log T_1}{c - c_1}$ where T T_1 = clotting times and c c_1 = heparin concentrations at two points on the line Since the coagulometer used measures clotting times to the nearest minute only the greatest error was on the normal clotting time Hence the line

The Clotting Time Response to Heparin in Vivo

As first reported by Howell [1] a single intravenous injection of heparin is followed by a rapid rise in the clotting time, and then by a more gradual but still rapid decrease to the normal value As seen from the relationship between heparin concentration and clotting time in vitro the rapid changes in clotting time at the higher values are due to the nature of the relationship between heparin concentration and clotting time Hence they do not reflect actual changes in heparin concentration in the blood and in order to use the clotting time to follow changes in heparin concentration in the blood it is advisable to plot the data as with the in vitro curves on semilog paper When this is done as in figure 2 it can be seen that

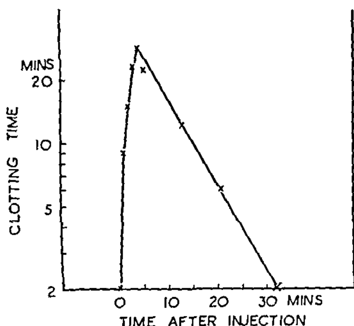


Fig 2 Effect of heparin in vivo on clotting time Single intravenous injection of 30 μ/Kg given at zero time

the anticoagulant effect of heparin disappears from the circulation in a relatively linear fashion at the concentration given by this dosage of heparin

In studying the clotting time response of the animal to injected heparin (fig 2) three different values are of interest These are (1) the time required for the clotting time to reach its peak value (2) the maximum clotting time reached and (3) the time required for the clotting time to return to normal or the duration of the hypocoagulable effect These three values will be considered separately

Time required for the clotting time to reach its peak value In figure 2 with a dose of 30 units Kg the maximum clotting time was reached in four minutes With a circulation time of 20 seconds one would expect that the heparin would have exerted its maximum effect within one minute after the injection Actually with larger

tween the normal clotting time and sensitivity to heparin Both hyper and hypo reactors were found with normal clotting times longer than the average value

The sensitivity of blood to the anticoagulant action of heparin depends (1) on the individual from whom the blood is taken and (2) on the technic used both for removal of the blood and for determination of the clotting time In the experiments reported in table 1 the dogs were under an anesthetic and the blood samples were taken from the exposed femoral vein Direct experiments showed that this procedure increased the sensitivity of the blood sample to heparin In a later series reported in table 2 the blood was taken by the usual venipuncture procedure through the skin The decrease in heparin sensitivity is shown by the decrease in the sensitivity value to the level 0 8–1 4 the median being 1 03 or a clotting time of 12 1 minutes for 0 5 units Hyp r and hyporeactors were not used for this series The series again demonstrates the individual variation even in a series of animals selected with regard to their response to heparin Likewise there is no indication from the normal clotting time of their response to heparin

The above demonstrates one factor which can change the sensitivity of the blood sample for heparin Other factors which can change this are the use of saline to moisten the syringe removal of the needle before transferring the blood to the tube for the determination surface coatings etc Finally the temperature at which the determination of clotting time is conducted markedly affects the sensitivity of the sample Heparin shows least anticoagulant activity at 20–25 C its activity increasing above and below this temperature range

While the great variation in the response of the clotting time to heparin due to changes in technic of venipuncture and determination of clotting times might suggest that the use of clotting times should be discarded as far as heparin is concerned actually the value of such determinations is greatly increased when these factors are appreciated and utilized As can be seen from figure 1 the relation between the clotting time and heparin concentration is such that measurable changes in clotting time can be obtained only over a rather narrow range of concentrations a considerable handicap in experimental studies on heparin However by suitably modifying the method it is possible for any desired range of heparin concentration within reason to be made measurable With proper control of these technical factors satisfactory reproducible results are obtainable with any given method

In using heparin both experimentally and clinically it would appear advisable to administer it on a weight or potency basis (i e in milligrams or units per kilogram) rather than on the basis of an arbitrary clotting time Since the same dosage is not necessarily required for all uses the dosage can be modified to the particular use It follows from the above discussion that the method for the determination of clotting time used to determine the duration of heparin action in the circulation as discussed below may be suitably modified to give measurable clotting times at this dosage level While the dosage for a particular purpose may be established for the average individual it may be advisable to modify it for those individuals whose blood proves to be either highly sensitive or relatively insensitive to the anticoagulant action of heparin

heparin was added at the end of the thirty minute period before the addition of calcium

The results for several animals are shown in experiments 1 and 2 of table 3 It is evident that after incubation with heparin the clotting times of the samples are much longer In order to avoid the decalcification the experiment was also conducted in another way It has previously been shown by Jaques Fidlar Feldsted and Macdonald[14] that coating glassware and needles with the silicone Dri Film

TABLE 2 —*Effect of Heparin on Clotting Time in Dogs**

Dog	Wt	In Vitro			In Vivo		
		Cl T† (no heparin) Min	Sensitivity [illegible]	Peak Cl T Min	Peak Cl T Mins	Duration Mins	Dose/Time
		Heparin—30 μ/kg					
A	29	2 8	1 34	6 9	26	32	90
C	30	2 6	1 18	10 2	28	32	90
E	34	3 0	1 68	9 6	>60	36	84
F	36	2 1	0 85	3 8	16	30	1 0
E	39	3 7	0 94	7 3	14	29	1 0
D	42	3 0	0 78	5 1	19	40	75
D	48	4 6	1 37	13 0	34	30	1 0
E	50	4 1	1 03	8 2	>90	25	1 2
D	52	4 5	0 96	8 7	22	24	1 3
E	56	3 7	1 24	8 7	42	39	76
D	57	4 1	0 99	8 2	24	30	1 0
Average		3 5	1 14	8 15	34	32	0 93
		Heparin—100 μ/kg					
C	33	3 5	1 34	77	>210	190	53
E	43	—	—	—	73	115	87
D	49	4 6	—	36	90	105	95
E	50	4 1	1 03	44	>240	125	80
E	54	2 0	2 30	400	>240	112	89
D	55	4 5	—	—	>110	95	1 05
Average		3 7				124	0 80

* Blood sample by venipuncture through the skin
† Cl T —Clotting Time

gives a surface which is apparently inert to the clotting system and that provided the blood sample is taken in such a way as to prevent contamination with damaged tissue such blood does not clot for several hours Five cubic centimeters of blood were removed from a superficial vein with a silicone coated syringe and needle The first and final 0 5 cc of blood were discarded and 1 cc portions of blood added to varying amounts of heparin in silicon treated tubes These were allowed to stand 10 minutes at 37 C The samples were then transferred with an untreated syringe

doses of 100 units/kg one usually finds that a ten to fifteen minute period is necessary for the clotting time to reach the peak value It is evident from this that the delay in developing the hypocoagulability of the blood following the injection of heparin is not due simply to the time required for mixing with the blood As discussed later an explanation of this delay has been found It also should be pointed out that this effect is observed only with doses of heparin which give clotting times that are still measurable With very large doses (1000 units/kg) complete incoagulability develops immediately

De Takats[3] has reported that the clotting time response to injected heparin is biphasic He noted a rise to the maximum within one minute of injection This was followed by an immediate return to the normal value which was then followed by the rise in clotting time usually observed No evidence of such a biphasic response is observable in the data reported in figure 2 nor have we observed any evidence of this in a series of dogs in which samples were taken for clotting times every minute after injection De Takats results were obtained on man However it is difficult to deduce any rational basis for such a phenomenon since as shown below there is essential even though not complete quantitative agreement between the clotting times obtained when the heparin is mixed with the blood in vivo and in vitro

The maximum clotting time It was concluded previously that the maximum clotting time obtained on the injection of heparin was the same as if a corresponding amount of heparin were mixed with the blood sample in vitro In the present investigation a more exact test of this hypothesis was conducted using the present methods In each case the effect of heparin concentration on clotting time was first determined in vitro by adding blood samples to various concentrations of heparin The results were then plotted on semilog paper to give a straight line and the slope of the line (heparin sensitivity value) is reported in the table Thirty or 100 units kg were then injected and the clotting time determined at frequent intervals as in figure 2 In table 2 is shown the maximum clotting times obtained for the injections heT clotting time for the same concentration of heparin added to the blood in vitro was determined from the in vitro experiment the blood volume being taken as 1 11th of the body weight This is likewise reported as the peak clotting time (in vitro) It can be seen that of fifteen injections the clotting time in vivo was always much greater than that obtained in vitro the value obtained from mixing in vivo being three to ten fold that in vitro

The only difference between the two series is that in the one case the heparin mixed in the body is in contact with the blood for the five to ten minutes required for the clotting time to rise to the maximum Quick[13] has reported that heparin shows an enhanced activity on incubation with an oxalated plasma system In order to test the effect on heparin activity of incubation with blood 10 cc of blood was drawn into 1 0 cc of sodium oxalate solution One cubic centimeter of oxalated blood was then added to each of a series of tubes containing 0 0 1 0 25 and 0 5 units of heparin and the tubes were allowed to stand thirty minutes at 25 C The tubes were then placed in the coagulometer equivalent calcium was added and the clotting time was recorded A control series of tubes was set up similarly but the

to the dosage In the previous study by Jaques[1] this was confirmed for when the dosage was divided by the time, the ratio obtained was approximately constant and was independent of the dosage level and of variations in individual animals The value obtained was 2 0 units Kg /min Shown in table 2 is the duration of the hypocoagulability for a series of animals receiving injections of 30 and 100 units/Kg In the present series likewise the ratio is constant The agreement is in fact considerably better than previously but the average value is 0 9 units/Kg /min Such a discrepancy is much too great to be due to experimental errors and merits further consideration

Several factors were different in the two studies First crude heparin (15 units /mg) was used in the first series while all later experiments were conducted with pure heparin Direct comparison of crude and pure heparin however showed no difference in the clotting time response elicited Secondly a series of doses (from 31 to 667 units/Kg) were used in the previous experiments while only two dosage levels (30 and 100 units/Kg) were used in the present series It is significant that in the previous study the values of the ratio for the 31 and 113 unit doses were 1 2 and 1 6 respectively compared with values of 1 9 to 3 4 (average 2 5) for doses of 200 units/Kg and over This result suggests that the value of the ratio changes with the dosage level of heparin Thirdly the clotting times were previously determined by the Lee and White method at 25 C with blood samples taken from the superficial vein through the skin and it was found that this method failed to detect heparin in concentrations in the blood lower than 0 3 units/cc On the other hand the present method detects concentrations of heparin as low as 0 10 units Hence at the time when the clotting time was normal by the first method heparin was still present to give a prolonged clotting time by the second method thus decreasing the ratio This factor appears to increase in importance with increasing sensitivity of the clotting time method used to detect the heparin Thus Jaques Charles and Best[16] reported data for the injection of 35 units/Kg in dogs The clotting time was determined with the coagulometer but blood samples were obtained from the exposed femoral vein thus further increasing the sensitivity In their data the hypocoagulability lasted from 40 to 80 minutes again providing a further discrepancy on comparison with the data reported in table 2 However this effect on this ratio of increasing the sensitivity of the test method for heparin could be predicted from the results reported by Jaques [1] of the effect on the clotting time of continuous injections of heparin He found that the rate of disappearance from the blood at blood levels below 1 unit per cc was a fun level so that increasing the sensitivity of the test would result in heparin in the blood for a relatively much longer time He likewise at blood levels greater than about 1 5 units per cc the heparin dis blood at a relatively constant rate This of course would result in proportionality between dosage and duration of hypocoagulabi during which blood levels below 1 5 units per cc could be detected comparison with the total period of hypocoagulability

It is evident then that the constancy of the ratio of dosage to

and needle to the coagulometer and the clotting times were recorded The values are shown in experiments 3 4 5 and 6 table 3 opposite I The control tubes in experiments 3 and 4 were set up identically and the heparinized blood then transferred to the coagulometer immediately without incubation In experiments 5 and 6 the control blood was added *to the silicon tubes incubated ten minutes* and then added to the heparin in the coagulometer tubes

It can be seen that irrespective of changes in the clotting time of the blood following incubation (zero heparin) there is a marked increase in the clotting time of the heparinized samples *This result was obtained consistently with marked differences* in technic and in the coagulability of the blood of the individual animal Evidently a certain period of time is required for heparin to combine with the com

TABLE 3 —*Effect of Incubation of Blood with Heparin on Clotting Time*

Hepa in Units		Clotting Time Mins			
		0	1	25	.50
1	C	1	6	45	95
	I	1	13	105	143
2	C	1	3	6	20
	I	1	4	25	40
3	C	2	8	18	70
	I	<1	7	25	90
4	C	2	10	28	84
	I	<1	11	58	106
5	C	1	4	9	18
	I	1	5	20	42
6	C	1	8	18	90
	I	1	7	43	140

C—Control or unincubated series I—Series with blood incubated with heparin
Experiments 1 and 2 with oxalated blood 3 4 5 and 6 with fresh blood in silicone

ponents of the clotting system before it can exert its maximum anticoagulant effect It appears reasonable to attribute to this factor both the discrepancy between the clotting times obtained on mixing the heparin with the blood in vitro and in vivo and also the lag in the rise of the clotting time to its maximum A great excess of heparin would accelerate the combination of heparin with the necessary factors of the clotting system and thus cause the disappearance of the lag As reported above, *it* was actually found experimentally that the lag phase disappeared with large do es of heparin

Duration of the hypocoagulability after heparin injections The duration of hypocoagulability represents the time during which the anticoagulant activity of heparin remains *in* the circulation Reed[15] reported that this time was directly proportional

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It is evident then that the constancy of the ratio of dosage to the duration of

hypocoagulability is more apparent than real However the ratio may have some value when used in a strictly empirical manner

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Waugh and Ruddick[4] have recently described a test for changes in the coagulability of the blood based on the effect of heparin on the clotting time when added in vitro De Takats test [3] on the other hand is based on the response when the heparin is injected Such tests are of value both as a possible indication of suspected thrombotic tendencies and as an indication of the dosage of heparin required The in vivo test as suggested by De Takats is essentially a heparin tolerance curve As is evident from the previous discussion this tolerance curve is established by two separate groups of factors The first is the coagulability of the blood itself This will determine both the value of the maximum clotting time and also at what level of heparin concentration in the blood it will be no longer possible to determine the residual heparin present by means of clotting time determinations The second factor consists of those processes whereby the heparin activity disappears from the circulation

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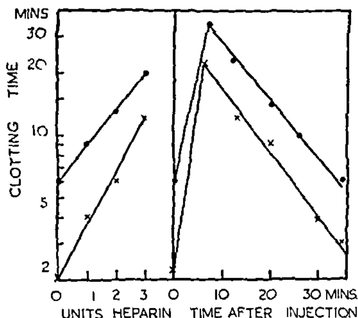


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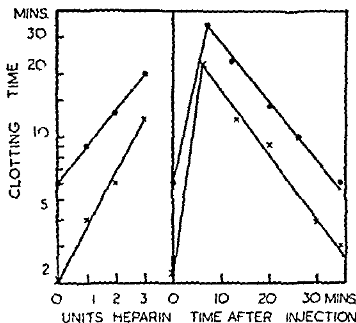


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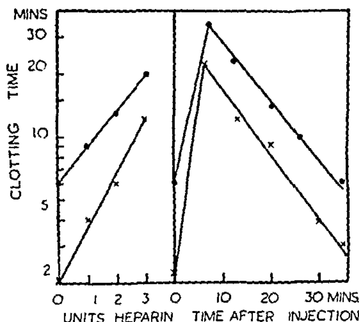


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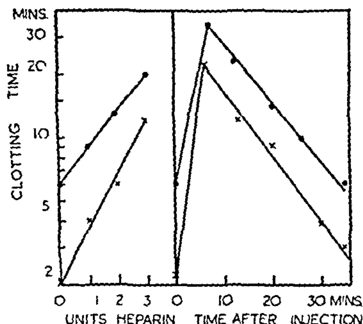


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iability under ether indicates that caution must be exercised in applying the test with this anesthetic However, the use of the test is possible following anesthesia with pentobarbital

The effect of treatment with india ink on heparin sensitivity Various investigators have suggested a relationship between heparin and the reticulo endothelial system Godlowski[17] originally reported that the injection of heparin resulted in a block age of the activity of the reticulo endothelial system as judged by the uptake of india ink Rigdon[18] has recently completely failed to confirm this On the other hand Knisely Bloch and Warner[19] have confirmed it microscopically Volkert[20] observed in rabbits that the injection of india ink caused the disappearance of 20 per cent of the antithrombin being the fraction in the blood considered by him to be heparin Also it prevented the rise in this fraction which he normally observed after the injection of protein The effect of india ink he concluded was due to an effect on the red cells whereby they could bind heparin

TABLE 4 —*Effect of India Ink Injections on the Heparin Tests*

Exp	Dog	Time after india ink injection	In vitro test		In vivo test Peak clotting time	Duration
			Clotting Time	Slope		
			min		m	m
35	E	preinjection	3.0	1.64	>60	36
		10	5.4	0.37	—	—
		3 hours 40	—	—	10	43
		6 days	3.7	0.96	14	29
56	E	preinjection	2.0	2.30	42	39
		4 hrs	3.7	1.24	12	23
37	F	preinjection	2.1	0.85	16	30
		4 hrs	2.8	0.96	4	20

A series of dogs was standardized and 5 cc of a 5 per cent suspension of india ink (Higgins) was given intravenously The response of the animals to heparin was then again determined The tests were conducted at various times after the india ink Immediately after the injection the blood was hypercoagulable to such an extent that it was not possible to conduct the tests In only one case (experiment 35) was it possible to obtain a satisfactory heparin curve immediately after the injection The india ink had disappeared from the plasma ten minutes after the injection as judged by the color of the plasma The most interesting and consistent effect of india ink was observed three to four hours after the injection Examples of this are shown in table 4 At this time a marked flattening of the response to heparin in vivo was observed as shown by the marked decrease in the peak clotting time The in vitro test showed some decrease in the sensitivity to heparin However this did not always appear to be responsible for the marked effect on the in vivo curve The duration of the in vivo test was decreased in some cases but not consistently with the decrease in peak clotting time The normal clotting time of the animal was lengthened The effect lasted partially as long as six days after the injection of the ink

The results resemble those of Volkert suggesting an interference in the reaction of heparin with the clotting system rather than either a change in the coagulability (which would be demonstrated by the in vitro test) or a change in the ability of the body to inactivate heparin (which would be demonstrated by the duration of the hypocoagulability in vivo). Rather there is an increase in the blood of nonspecific factors which interfere with heparin action. Since blockade of the reticulo-endothelial system with india ink does not increase the duration of hypocoagulability after heparin injections the reticulo-endothelial system is probably not involved in the disappearance of heparin from the circulation.

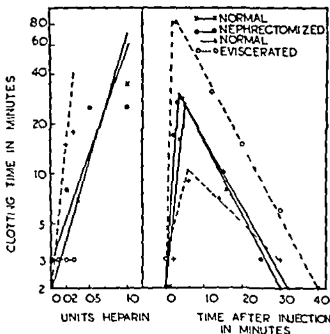


Fig 4 Effect of nephrectomy and evisceration on the response to heparin. Left hand figure in vitro right hand figure in vivo. x——x animal under pentobarbital anesthesia before operation ●——● same animal after removal of the kidneys +——+ animal under pentobarbital anesthesia O——O same animal after evisceration

Effect of nephrectomy and evisceration on heparin sensitivity. As shown in figure 2 the anticoagulant action of heparin disappears rapidly from the circulation. Howell suggested that heparin was excreted by the kidneys and evidence in favour of this view has been obtained by Wilander[1] and Copley and Schnedorf. However Jaques[1] and Reinert and Winterstein[2] did not detect any excretion in the dog or in man. As a more direct test of this problem both kidneys were removed from four dogs under pentobarbital anesthesia. These animals had been carefully standardized on heparin and the heparin sensitivity was again tested immediately after the operation while the animals were still under the anesthetic. The results of one experiment are shown in figure 4. It can be seen that there is no change in the response

to heparin either in vitro or in vivo While the points of the in vitro curve do not lie on the straight line as closely as is usually the case there is no significant difference between the values before and after operation One animal showed an increase in clotting time and one showed a decrease after the operation In these experiments the curves after operation were simply displaced by a corresponding amount, in dicating that there had been no change in sensitivity to heparin in vitro or in vivo

Since the kidneys do not appear to be directly responsible for the rapid disappear ance of hypocoagulability after heparin injections the gastrointestinal tract was similarly investigated Three dogs were carefully standardized for the heparin test under pentobarbital anesthetic The gastrointestinal tract and related organs in cluding the spleen were then removed Since removal of the liver is followed by a rapid fall in prothrombin introducing a further difficulty in interpreting the test care was taken not to damage the liver or hepatic artery After completion of the operation a good pulse was observed in the hepatic artery The response of the animal to heparin was determined one hour and three hours after the operation Glucose was given postoperatively to two animals but not to the third No differ ence was observed in the response of the three animals

The results obtained in one animal are shown in figure 4 After the operation the animal s blood was hypercoagulable as shown by the lack of response to heparin added in vitro The response in vivo was also changed A much greater peak clot ting time was obtained This was to be expected since in removing the viscera we also removed a large proportion of the effective blood volume so that the heparin concentration of the 30 unit kg dose was proportionately much greater when calculated as units/cc of blood However the clotting time returned to normal in 35 minutes compared with 30 minutes before operation It is evident that the gastrointestinal tract likewise is not responsible for the disappearance of heparin from the circulation

Summary

1 The relationship between clotting time and heparin dosage has been studied in the dog

2 On the addition of heparin to blood in vitro a linear relation is found between heparin dosage and the logarithm of the clotting time obtained The sensitivity of the blood sample to the action of added heparin is influenced both by the indi vidual (coagulability of the blood before withdrawal) and by the technics of with drawal and of determination of the clotting time It is indicated that alterations in the latter may be used to extend the range of measurable hypocoagulability due to heparin Incubation of heparin with blood for ten minutes increases its anti coagulant effect

3 When moderate doses of heparin are injected intravenously five to fifteen minutes are required for the clotting time to reach a maximum No evidence of a biphasic response was obtained The maximum clotting time obtained is greater than it is with the same amount of heparin added to the blood in vitro due to the effect of incubation of heparin with blood on its anticoagulant activity The in

terval required for the clotting time to return to normal is quite short and with a given dosage is constant with different animals Factors influencing the relation between duration of hypocoagulability and dosage are discussed

4 A test has been devised to determine the sensitivity of the animal to the anti coagulant action of heparin The clotting time response to certain concentrations of heparin added to the blood in vitro is determined A fixed dose of heparin is then injected intravenously and the clotting time response is again determined The response in vitro measures the sensitivity of the clotting system to heparin while the in vivo response when interpreted in the light of the in vitro response meas ures the ability of the body to remove heparin from the circulation

5 By means of this test it has been determined that anesthesia with pentobar bital decreased the coagulability of the blood urethane had no effect on coagulabil ity while the effect of ether was variable The injection of india ink and eviscera tion caused a hypercoagulability while removal of the kidneys had little effect

6 When the sensitivity of the blood to the anticoagulant action of heparin was tested during these procedures pentobarbital and nephrectomy had no effect ether caused an increase in sensitivity urethane a decrease The injection of india ink and also evisceration markedly decreased the sensitivity of the blood to the anti coagulant action of heparin

7 Anesthesia with pentobarbital ether or urethane the injection of india ink removal of the kidneys or removal of the gastrointestinal tract had no effect on the duration of heparin action in the body

ACKNOWLEDGMENT

We are greatly indebted to Professor C H Best for his interest and encouragement in these studies and to Dr J Markowitz for performing the experimental surgery for us The study was supported by a grant from the John and Mary R Markle Foundation

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PART V

LEUKEMIA

IS IT POSSIBLE TO TRANSMIT OR ACCELERATE THE DEVELOPMENT OF MOUSE LEUKEMIA BY TISSUE EXTRACTS?

By J ENGELBRETH HOLM M D

DURING the last ten years it has been reported from various laboratories that the injection of certain tissue extracts has been followed by accelerated devel opment of spontaneous leukemia in mice the disease presenting an increased inci dence and (or) an earlier appearance in the experimental animals than in the untreated controls Although these observations lack satisfactory confirmation it seems desirable to review them here and to record some supplementary investi gations bearing on the same problem

In the Year Book of the Carnegie Institute New York for 1937 MacDowell and collaborators described experiments in which monthly injections of embryonic tissue extract into mice of the strain C 58 (with a 90 per cent leukemia incidence) were followed by development of the disease in all of the 60 experimental animals at an earlier date than in the controls belonging to the same litters The results were not reported in detail nor was it stated whether the test has b en rep ated Gorer who tried to confirm this finding states merely Inoculations of embryonic tissue have had no noticeable effect on either the albino or the black leukemia

In 1938 Engelbreth Holm and Frederiksen believed they had transmitted mouse leukemia to young animals of the strain Aka by means of a cell free extract of leu kemic organs from mice of the same strain The extract was prepared under anaer obic conditions reduced in a cysteine-cobalt sulphate system as described by Pirie and Holmes Injection of the extract was followed by the development of leukemia in 8 experiments out of 9 totalling 36 mice out of 179 The tests were carefully controlled in various ways Thus *aerobically* prepared extracts showed no effect in 5 experiments including 120 animals Further a minimum of 1000 cells was found to be necessary to secure a take in the ordinary way it therefore seemed impos sible that the takes in these experiments could have been due to presence of sufficient intact cells in the extract since the latter had been centrifuged twice for fifteen minutes at 3 000 r p m As a most deplorable fault it must be noted that the extract was not filtered in these tests Engelbreth Holm later (in 1942) expressed the view that the findings in these experiments might have been a question of accel eration of spontaneous leukemia rather than of a transmission of the disease

MacDowell and his collaborators (1939) tried to repeat the observations of Engelbreth Holm and Frederiksen without success Still more confusion however was brought into the matter when after control injection of the medium used for reduction (a cobalt sulphate cysteine solution) MacDowell found leukemia devel oping in 17 out of 20 mice only twenty six to thirty eight days later Repetition of this experiment gave negative results

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These investigations have been aided by grants from the Anders Hasselbalch Leukemia Fond the Konig Christian den Tiendes Fond and from the National League for Combating of Cancer

cells (a finding which was to be expected beforehand) leaving it thus still more improbable that our takes or accelerated cases in the experiments of 1938 could have been due to the presence of intact cells in the extract solution

In 1946 however a paper was published by Silber in Russia describing investigations which may possibly throw some light on these obscure questions though it is necessary to await confirmation of the findings before reaching a final opinion According to Silber sarcomas induced in mice by 1 2 5 6-dibenzanthracene are transmissible by means of Berkefeld filtered extracts of tumor tissue which has been treated according to the method introduced by Engelbreth Holm and Frederiksen (dissection in a closed chamber filled with carbon dioxide and suspension in a cysteine-cobalt system in order to prevent oxidation)

In 4 out of 5 experiments Silber had takes in a total of 18 out of 114 animals He makes it clear however that the takes occurred only when the substance had been prepared from very young tumors (incipient sarcomas) and further that the experimental animals required to be sensitized by subcutaneous injection of 0 5 cc of an oily solution of Dibenzanthracene containing 1 mg of this substance in one liter of vegetable oil This injection was administered 1–– weeks before the test Without this sensitization no takes were seen

TABLE 1

Number of leukemic cells	Suspended in saline "Takes" number of mice	Suspended in cobalt cysteine solution "Takes" number of mice
1 000 000	1 2	3 5
100 000	5 5	0 5
30 000	[illegible] 5	0 5
5 000	0 5	0 5

An observation by Duran Reynals in fowl sarcomas may also prove of interest in this discussion Duran Reynals has pointed out that the virus of fibrosarcomas are detected more frequently at the age of 5 to 10 months than in younger or older fowls

Whether or not these findings have any bearing on transmission of leukemia in mice cannot yet be decided In our experiments no attention was paid to the age of the donor animal or to that of the tumor tissue nor was it possible to pay regard to such changes of character as might have taken place in the inbred mouse strains during the passages

Attempts have further been made to repeat Gorer s experiments mentioned above In our experiments different tumors were inoculated into mice belonging to three different strains in which the tumors used did not take Inoculation was made subcutaneously and repeated every two weeks (see table —) one half of each litter being left untreated as controls The three strains employed were the strain Aka the strain dlb and the strain Street The strain Aka has a spontaneous leukemia incidence of 57 per cent in the strain dlb (subline of the Little Dba) leukemia will develop in 1 per cent and mammary carcinoma in about 40 per cent and in the strain Street leukemia incidence is 1 per cent the incidence of mammary carcinoma

Finally Gorer in 1939 reported a somewhat analogous observation Inoculation into mice of a nontaking sarcomatous tissue appeared to increase the leukemia incidence from 6 to 39 per cent in all animals and from 2 to 46 per cent in the males Reinoculation brought about a still greater rise in incidence Gorer inoculated a sarcoma from an albino strain into a black mouse strain After repeated inoculations leukemia developed in 7 out of 10 mice whereas the spontaneous leukemia incidence was only 6 per cent Gorer found that the leukemia did not develop until about one year after the inoculations i e probably at the same age at which the disease will appear in untreated mice

In an attempt to explain the results of our original experiments (Engelbreth Holm and Frederiksen) new investigations along the same lines were performed during the years 1938 42 Our efforts however were no more successful than those of MacDowell We did not observe any acceleration of the leukemia in mice belonging to the strain Aka after injection of an extract of leukemic organs prepared under anaerobic conditions Repeated injections of such extracts were made in 25 mice belong to the strain Aka a total of 17 injections being administered at intervals of two weeks For control purpose extracts of normal organs were injected into brothers and sisters of the experimental animals but no effect was seen in either of the two series

Likewise MacDowell s experiments with the cobalt sulphate cysteine solution were repeated Forty eight Aka mice were given 1 cc of the solution the mice at the beginning of the experiment were one month old and the injections were administered every two weeks until they died spontaneously In 23 out of these 48 mice leukemia developed but among their 44 untreated brothers and sisters 23 cases of leukemia were found as well the treatment therefore had had no evident effect

No more did we succeed in accelerating other tumor types leukemia and mammary carcinoma of the strain dlb These experiments comprising 38 and 15 mice respectively were equally negative

The most natural way in which to explain these rather capricious and mostly negative results was to assume that despite the precautions taken the spun extract in our 1938 experiments did contain a number of intact cells sufficient to secure takes this explanation still failed to account however for the fact that administration of *aerobically* prepared extracts did not give any takes In spite of numerous unsuccessful attempts to repeat our 1938 observations and in spite of the controls having indicated that a few intact cells in the injected substance could not explain the development of leukemia I initiated one more experiment in order to exclude the possibility that cells when suspended in the cobalt sulphate cysteine solution were more capable of taking than when suspended in a sodium chloride solution as in the original control experiments

Known numbers of leukemic cells suspended partly in normal saline and partly in a cobalt cysteine solution were accordingly injected into series of mice The result may be seen in table 1

No explanation was achieved by these experiments as suspension in the reduction solution had a definite effect in suppressing the taking capability of the

Englebreth Holm and Frederiksen thought that they had accelerated the development of leukemia or transmitted the disease by means of an anaerobically prepared extract of leukemic tissue These findings however have remained refractory to reproduction in spite of repeated attempts made by MacDowell and ourselves

MacDowell observed acceleration of leukemia in one experiment by means of a cysteine-containing suspension but neither he nor we have been able to repeat this observation

After transplantation of a nontaking tumor tissue Gorer achieved an accelerated development of leukemia Despite several attempts to repeat these experiments, however we have not succeeded in confirming Gorer s results

How these discrepant findings are to be explained is still obscure The various control series have been adequate and no experimental faults which might have produced the positive or negative results have been detected Most peculiar is the fact that the three positive series of experiments quoted although mutually different show one common feature viz the injection of rapidly growing tissue or tissue extracts that is of homologous or heterologous tumor tissue and of embryonic tissue extract

It is a most fascinating thought that in these experiments we may be approaching a factor capable of accelerating tumor development but undeniably there must still be a number of factors escaping our control

Transient changes of disease conditions in the mouse strains used can probably be excluded to judge from the control series Certain more recent experiments however may possibly throw light on these questions Silber claims to have transmitted sarcomas in mice by means of cell free filtrates using the same technic as we did in our earliest experiments but he points out that only filtrates from quite young tumors have any effect and Duran Reynals has shown that detection of virus in fowl sarcomas most easily will be successful in certain age groups younger and older animals offering more difficulties

It is premature to attempt to assess the importance of these experimental results to mouse leukemia but they seem to call for a re-examination of the relevant problems on a wider basis

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being 25 per cent The transplanted tissues in question were a mammary carcinoma from dlb mice a leukemic tissue from Aka mice and a squamous cell carcinoma *from the strain Aka which had been transferred through* several passages (see Engelbreth Holm 1944)

Further details of the experiment may be seen in table 2 The treatment did not increase or accelerate tumor development, and the results of Gorer s investigation were therefore not confirmed Unfortunately however owing to a fulminating epidemic all the animals died when 17–19 months old, and it is impossible to decide how many tumors might have developed if the mice had not succumbed prematurely Nevertheless since tumors occurring in these strains will generally develop spontaneously from the age of 10 to 12 months the climax being at about 15 months it was clear that the treatment did not accelerate tumor development both the experimental and control animals presenting only a few tumors

TABLE 2

Tumor tissue from	Inoculation made into	Number of inoculations	Age of mice at the end of experiment
Mammary carcinoma dlb	24 Aka mice	3	17–19
	53 Street (24 ♂ 29 ♀)	5	
Leukemic tissue Aka	55 dlb (34 ♂ 21 ♀)	6	18
	52 Street (30 ♂ 2[illegible] ♀)	6	18–19
Squamous cell carcinoma Aka	49 dlb (28 ♂ 21 ♀)	6	17–18
	54 Street (30 ♂ 24 ♀)	6	1

The result in each instance was No effect upon tumor development

We have thus been unable with the strains used in these experiments to repeat Gorer s finding that inoculation of heterologous tumor tissue can bring about an increased leukemia incidence

DISCUSSION

A series of experiments is reviewed although largely supporting each other they have proved inaccessible to direct reproduction The various positive investigations originally indicated that development of spontaneous leukemia in inbred mouse strains is accelerated after the injection of embryonic extract or *leukemic* tissue extract or after inoculation of heterologous tumor tissue

MacDowell and his collaborators succeeded in accelerating the leukemia incidence after administration of embryonic tissue extract Gorer did not succeed in reproducing these experiments but he gives few details of his negative result to which indeed he seems to ascribe but a limited importance

Englebreth Holm and Frederiksen thought that they had accelerated the development of leukemia, or transmitted the disease by means of an anaerobically prepared extract of leukemic tissue These findings however have remained refractory to reproduction in spite of repeated attempts made by MacDowell and ourselves

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IS LEUKEMIA A DISEASE OF THE RETICULO ENDOTHELIAL SYSTEM?

By Bruce K. Wiseman, M.D.

AT THE twenty second year milestone following discovery of a rewarding treatment of pernicious anemia by Minot and Murphy, there is little doubt that the problem presented by leukemia is more important than any other in the field of hematology. Whether the increasing incidence in this disease is actual or only apparent due to more refinements in diagnosis (especially the widespread use of bone marrow biopsy) and more physicians interested in hematology is debatable. In any case the frequency with which this diagnosis is made is undoubtedly rising.[1] This fact is highlighted by the observation that little or no real progress has been made in pathogenesis or treatment, since with unimportant exceptions the efficiency of treatment by blood transfusions and x ray therapy is still supreme (and unsatisfactory) and the prognosis for longevity remains unchanged. These facts suggest that a fresh, if not new, point of view with respect to this disease would not be undesirable. The present observations in a series of clinical and hematologic studies of monocytic leukemia may suggest an important role of the reticulo-endothelial system in the mechanism of the production of leukemia.

The Reticulo Endothelial System

Although ameboid cells in the connective tissues, distinct from the blood cells, were described as long ago as at least 1863, it remained for Aschoff in 1913, working with vital staining methods using lithium carmine, to recognize that the phagocytic cells described by various cytologists under varying names were widespread throughout the body, forming a system of cells.[3,4] Almost at the same time hematologists were struggling with the problem of the identity of the monocyte, thought to be a white blood cell with exceedingly well developed powers of phagocytosis but resembling the neutrophilic leukocyte on the one hand and the lymphocyte on the other. These conflicting observations were resolved by Schilling Torgan[5] by establishing that the monocyte is a separate cell type. This was the general situation until 1925 when abundant evidence began to accumulate from the study of inflamed tissue and especially from tissue culture technics[6–10] that monocytes and clasmatocytes were capable of transformation from one to the other (table 1). Recently, in our laboratory, this transformation has been convincingly demonstrated by Houghton[11] with a single cell tissue culture technic. This observation has been further strengthened by the identification of transitional types of mononuclear phagocytic cells in human blood which, when stained supravitally, have characteristics of both monocytes and clasmatocytes (see also fig. 6).

At present, therefore, it seems to some of us rather convincingly demonstrated that the monocyte is a derivative of the reticulo-endothelial system and that this system, having a blood as well as a tissue component, greatly exceeds in extent and

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importance than which was originally indicated by the concept of Aschoff and Kiyono Monocytic leukemia therefore may be regarded as fundamentally and in fact as a leukemic reticulo-endotheliosis Monocytic leukemia might therefore furnish a valuable approach to the study of the various reactions and potentials of the reticulo-endothelial system of cells when under intense stimulation

Reactions of the Reticulo Endothelial System Terminating in Monocytic Leukemia

During the past seventeen years in our clinic at Columbus 192 cases of monocytic leukemia have been studied Many of these have shown the most unusual cytologic reactions in our entire experience with the blood dyscrasias of all types This has recently been the subject of comment [1-13] Cases initially appearing to be

Table 1 —*Historical Development of Identity and Relationships of Monocyte Clasmatocyte and Fibroblast*

Changing concepts of the separate identity of the monocyte are shown in the left panel of the clasmatocyte in the right panel The bottom horizontal strip indicates that under proper environmental conditions monocyte clasmatocyte and fibroblast may revert *from* the one to the other

Monocyte	Clasmatocyte
Blood	Connective Tissue
Transitional Neutrophile (Ehrlich Naegeli)	Clasmatocyte (Ranvier 1891)
Neutrophile does not contain azur granules (Michaelis & Wolfe 1902)	Macrophage separated from microphage (Metchnikoff 1892)
Independence of Monocyte & Neutrophile (Pappenheim & Ferrata 1911)	Adventitial Cells (Marchand 1890)
	Polyblast (Maximow 190)
Monocyte a separate cell type (Schilling Torgau)	Clasmat Macrophage Adventitial cell and Polyblast identical (Goldman 1909)
Monocytic leukemia described (Schilling Torgau & Reschad 1913)	Histiocyte & R E System (Aschoff & Kiyono 1913)

Monocyte ⇄ Clasmatocyte ⇄ Fibroblast

Tissue Culture Lewis Houghton etc 1925 46

myeloid or lymphatic leukemia but terminating as classic monocytic leukemia have been seen in addition to those which showed approximately equal numbers of all three cell types throughout the entire course of the disease Instances in which there was an early stimulation of megaloblasts have been observed Polycythemia vera coexisted in one case In several instances long remissions[14] with near normal hematologic recovery occurred at a time when with marked anemia and thrombocytopenia existing the disease was thought to be far advanced Examples of a few of these unusual hematologic reactions will be given in brief summarization

Case Reports

Ca (Fig 1) This patient a colored male age 28 was first seen on December 30 1946 presenting marked generalized adenopathy and splenomegaly The lymph nodes were exceedingly hard upon

palpation and the spleen quite nodular but also very hard Repeated bone marrow aspirations from the sternum failed to show many free cells the bone marrow content being chiefly reticulum cells and a few monoblasts Bits of solid bone marrow tissue however consisted almost entirely of reticulum cells An occasional megacaryocyte was seen and a fair sprinkling of myeloid cells in all stages of maturation Lymph node biopsy showed complete loss of architecture the cellular content being composed almost

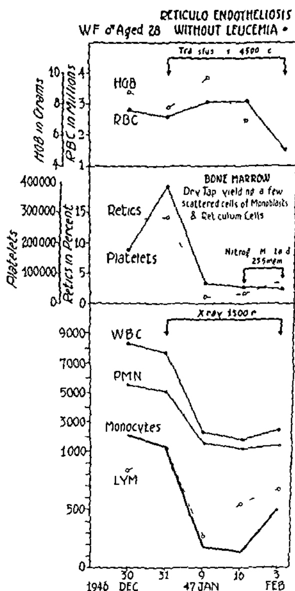
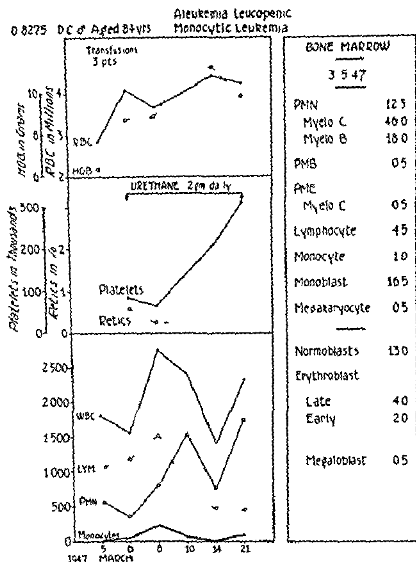


Fig 1 Graph of the hematologic reactions in a patient with pure reticulo endotheliosis Case 1 of text White cells in semi logarithmic scale

entirely of reticulum cells and monoblasts Reference to figure 2 shows increasing difficulty in the supply of circulating blood elements even before the application of the nitrogen mustard and deep x ray therapy which incidentally resulted in no visible decrease in the size of the adenopathy or splenomegaly

This case is shown as an instance of reticulo endothelial hyperplasia with very little tendency to do other than reduplicate its own type of cell This therefore

would be an instance of almost pure reticulo-endotheliosis and is to be contrasted especially with the following case



F g 2 Graphic representation of the blood findings in a patient with aleukemic reticulo-endotheliosis Case 2 of text The differential cell count of the bone marrow as obtained by the aspiration technic is shown in the vertical panel on the right Nucleated red cells listed refer to the number encountered in counting 100 white blood cell

Case (Fig 2) This patient a white male aged 84 years has been ill for sixteen months during which time his clinical and hematologic state has varied little He has received blood transfusions about once a month to maintain his red cell count There is no apparent physical deterioration and he goes about in the same fashion as almost any man of the stated age Figure 2 shows the hematologic record

for a typical month (March 1947) during which time he received urethane Although this medication apparently improved the levels of blood platelets and neutrophiles it was discontinued because the patient definitely felt worse when he was on this form of treatment Of importance to the present discussion is the fact clearly shown on this graph that the level of monocytes in the blood are very low with no *immature forms present at all* while the bone marrow constantly shows a small but definite percentage of monoblasts which does not vary appreciably from time to time

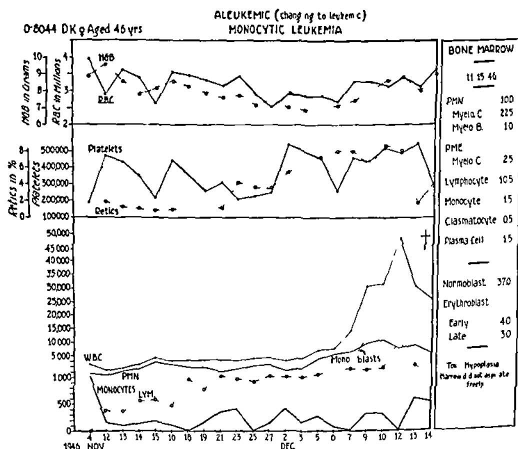


FIG 3 Graphic representation of the blood findings in a patient in which transition from aleukemic to leukemic monoblastic leukemia occurred White cells in semi logarithmic scale Case 3 of text The differential cell count of the bone marrow in the aleukemic phase is shown in the vertical panel on the right Nucleated red cells listed refer to the number seen in counting 50 white blood cells

This case illustrates a minimal reaction of the reticulo endothelial system in the direction of producing monoblasts with little tendency for maturation to monocytes This is reflected in a clinical course that is unchanging paralleling the stationary character of the hematologic reaction

Case 3 (Fig 3) This patient a white female aged 46 years illustrates the transition from an aleukemic state in which no qualitative or quantitative changes in the blood monocytes could be detected to a *frank monoblastic leukemia* Also illustrated is the deceptiveness of attempting to interpret the bone marrow findings as obtained by the aspiration technic when the marrow does not aspirate freely Re

peated samplings of the marrow were attempted from the sternum but only a few drops of acellular fluid were obtained on each occasion In this material no free monoblasts and no increase in monocytes were obtained as shown in figure 3 (right panel) Monoblasts first appeared in the blood seven days before death but monocytes were never increased

Case 4 (Fig 4) Demonstrated here is a case of monoblastic leukemia in which during the early phases of the disease an appreciable number of megaloblasts were found in the bone marrow when monoblasts were not apparent in this tissue When leukemia first became clearly evident small numbers of monoblasts began to appear in the marrow but megaloblasts were no longer to be found Later large numbers of monoblasts were present in both blood and bone marrow

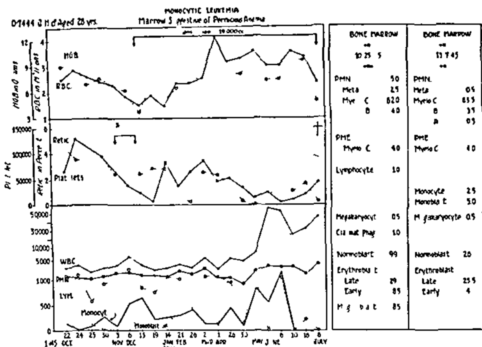


Fig 4 Hematologic graph of a patient with monoblastic leukemia in which megaloblasts and increased numbers of early erythroblasts occurred in the bone marrow during the early course of the disease Case 4 of text Typical bone marrow findings are given during the early phases (first vertical panel on right of graph) and late phases (second vertical panel) of the leukemia Nucleated red cells shown indicate number seen in counting 100 white blood cells White cells plotted in semi logarithmic scale

The data in this case suggest a low grade stimulation of the reticulo-endothelial system in the early phases of which pathologic megaloblasts were the first abnormal free cells to appear in proximity to reticulo-endothelial tissue later the stimulus resulted in more directional changes in terms of formation of monoblasts

Case 5 (Fig 5) This case of monocytic leukemia is remarkable because of the coincident polycythemic levels of red blood cell The patient had a large spleen (extending to the level of the umbilicus) which was removed at another hospital the tissues being unfortunately lost When first seen in this clinic the usual clinical signs of polycythemia vera were present i e cherry red mucous membranes liver like tongue distended dark retinal veins chronic conjunctivitis etc There was no adenopathy

The initial blood examination (fig 5) showed high levels for all the circulating blood elements including reticulocytes However the only pathologic white cells found were monoblasts although young and mature monocytes were distinctly plentiful Radiation therapy with radioactive phosphorus as shown

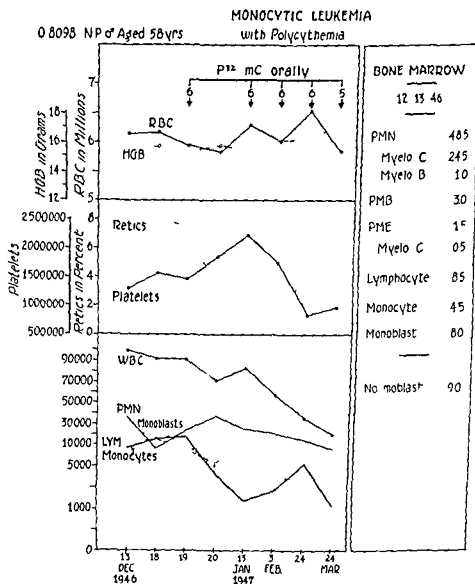


FIG 5 Hematologic graph in a patient with monoblastic reaction occurring coincidently with the blood and clinical findings of polycythemia vera Case 5 of text Bone marrow count is shown in the vertical panel on the right Nucleated red cell elements are given as the number encountered in counting 100 white blood cells White cells in semi logarithmic scale

presumably has decreased the monoblasts in the blood almost to the vanishing point so that now the patient shows little else than the classic hematologic and clinical signs of polycythemia vera

We interpret these bizarre hematologic events to be the result of increased stimulatory effects upon the reticulo-endothelial system with dominate effect upon the

intersinusoidal reticulo-endothelial system capillaries (red cell-forming precursory tissues) of the marrow producing increased numbers of mature erythrocytes Elsewhere this stimulus results in a monocytic response resembling the average

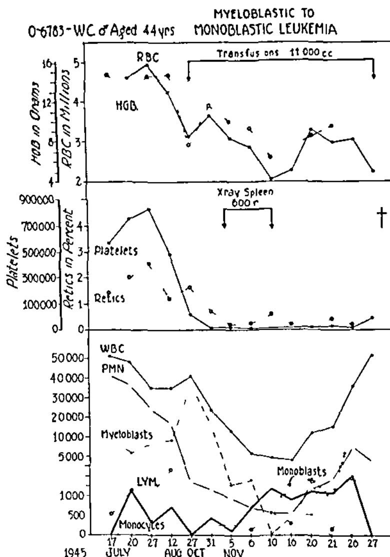


Fig 6 Graphic representation of the blood findings in a case of myeloblastic leukemia later terminating as monoblastic leukemia Case 6 of text White cells in semi logarithmic scale

case of monocytic leukemia except for the unusually favorable response to radiation therapy

Case 6 (Fig 6) This patient initially presented with the peripheral blood and bone marrow findings of myeloblastic leukemia As shown in figure 6 neutrophilic leukocytes constituted 80 per cent of the circulating level of 50 000 white blood cells indicating that our diagnosis of the immature cells present

at that time as myeloblasts and not monoblasts was probably correct Subsequently and coincidently with radiation therapy as shown the myeloid reaction completely disappeared to be replaced by a blood and bone marrow picture of monocytic leukemia which persisted until death Autopsy findings in this and the preceding cases were those usually noted in monocytic leukemia described in a previous publication from this[15] and other laboratories [16] There is some evidence here that myeloblastic leukemia may

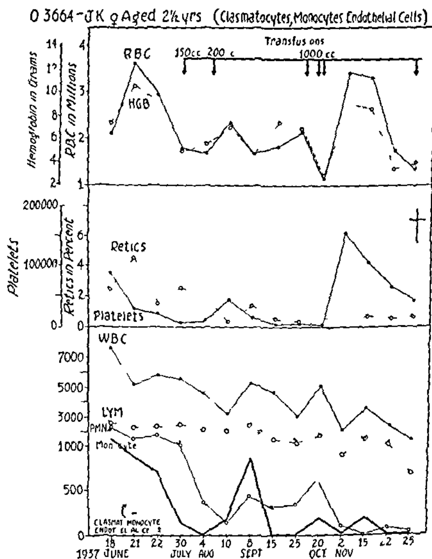


FIG 7 Clasmatocytic leukemia characterized by many transitional phagocytic mononuclear cells Hematologic graph of a case of reticulo endothelial leukemia Case 7 of text White cells in semi logarithmic scale

be one manifestation of reticulo endothelial disease If the myeloblasts did not arise originally from reticulo-endothelial stimulation it is difficult to understand why the proliferation of these cells did not persist with the advent of the monocytic reaction

Cases similar to the above in which the initial cell stimulation consisted of lymphocytes with immature lymphoid elements present and others in which im

mature elements of all three types of white blood cells were increased in approximate equal proportions, have been previously described and reported from this laboratory[16] and need not receive additional emphasis in this communication

Case 7 (Fig 7) This patient furnishes through the observed hematologic reactions during her illness additional evidence that the reticulo-endothelial system may undoubtedly undergo stimulatory changes resulting in a leukemia of reticulo-endothelial cells In this chart only morphologically classic monocytes are labeled as such represented by the heavy solid line However the dotted line of the graph represents phagocytic cells many of which had predominating monocytic characteristics as well as cells that were definitely clasmatocytes and endothelial cells There is little doubt that this was a leukemia of reticulo-endothelial elements in which monocytes participated as one of the pathologic cells Blood cultures were sterile and there was no evidence of bacterial endocarditis or other sepsis

Discussion

Doubt that monocytes are derivatives of the reticulo-endothelial system and that monocytic leukemia is therefore not a disease of this system of cells has often been expressed,[17] chiefly because reticulo-endothelial hyperplasia is not always demonstrable in this disease It should be pointed out however that numerical increase in the reticulum and specific endothelial cells probably will not be apparent unless *maturation of these elements is obstructed* That is to say when cell division and maturation occur uninhibited hyperplasia of that cell type often is not apparent by microscopic examination of the tissue in question the numerical increase is noted only in the end state cell An excellent example of a tissue reaction supporting this statement is furnished by almost universally accepted observations in pernicious anemia During the phase of relapse megaloblastic hyperplasia with the separate power of division of the cell intact is outstanding in the near absence of maturative principle When the maturative principle is supplied however megaloblasts rapidly disappear so that within forty eight hours and thereafter no greater number of megaloblasts can be found in the bone marrow than is apparent in a normal resting marrow Within seven days however mature red blood cells are being supplied to the circulation in maximum numbers i e only the end cell product is visibly increased although little doubt can be entertained that these new red cells are taking origin primarily from the megaloblasts There is little reason therefore to demand visible evidence of reticulo-endothelial hyperplasia in monocytic leukemia to satisfy the hypothesis that in this disease the monocytes take origin from the reticulo-endothelial system *In a blood cell strain it is only the cell in the end stage of maturation that regularly shows appreciable and visible quantitative increase under conditions of stimulation not the precursor cells*

If this statement is accepted it follows that there is no valid reason to discount the distinct possibility that leukemias of all cell types are primarily diseases of the reticulo-endothelial system The case studies cited in the foregoing part of this paper offer some evidence that under an unknown type of stimulus to the reticulo-endothelial system (as indicated by the advent of monocytic leukemia at some phase of the disease) there may be formed large numbers of myeloblasts lymphoblasts and even megaloblasts It is possible in the cited cases that hyperplasia of one or another of the types of cells occurred because maturative substance for that

cell type was temporarily deficient Eventually, the body resources were able to mobilize adequate quantities of maturing substances for this cell type the final failure coming in inability to supply sufficient cell maturing factor for monocytes thus in the end determing the death of the individual from monoblastic leukemia

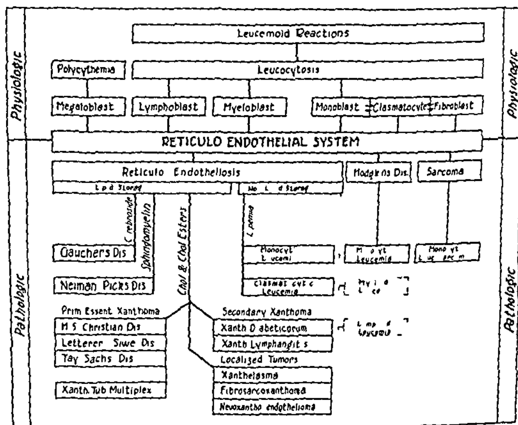


Fig 8 Diagrammatic representation of the relationship between the reticulo endothelial system normal blood formation (upper one third of chart) and pathologic reactions producing disease (lower two third of chart) This chart expresses the view that neoplasm of the reticulo-endothelial cells (labeled sarcoma) gives a different reaction than that of the bulk of the diseases of the reticulo endothelial system (labeled reticulo-endotheliosis) the latter being characterized by among other things disturbances in lipid metabolism Hodgkin s disease is shown as a third form of disturbance of reticulo endothelial tissue It is possible that sarcoidosis (not shown) is a fourth variety of reticulo endothelial disease Myeloid and lymphoid leukemia are indicated in broken line as possibly also primary disease of reticulo-endothelial cells as suggested in text

This discussion leads to the following suggestions of a possible mechanism for the production of leukemia

1 Leukemia *irrespective of cell type may be the result of unknown stimula*tory effects upon the reticulo-endothelial system

2 The type of leukemia observed may be determined by the failure of the body to supply specific maturative substance or substances at one or more phases in development in that cell strain in quantity to keep up with the particular intensity

of reticulo-endothelial stimulation operating at that time in that individual organism

3 Specific cytologic maturative substances may be multiple in types and chemical identity and failure of supply of one type may not necessarily prejudice adequate supplies of another type The chain breaks at its weakest link

4 The bizarre varieties of leukemia regularly seen in all hematologic laboratories may result from multiple mixed failures of maturation factor varying as to type specificity and as to degree

This explanation of the production of leukemia although admittedly speculative satisfactorily accounts for many if not all of the puzzling features regularly encountered in patients with this disease by using only one basic mechanism without recourse to multiple theories The concept of specific cell maturation substances is cytologically correct and the existence of one such substance for megaloblasts has been proved In addition to the need for more information relating to other maturative factors more facts are needed with respect to influences that are stimulatory to the reticulo-endothelial system Particularly in this regard is there need for additional study of lipid metabolism and the influence of lipids upon this system of cells[15 18–20] (fig 8)

Summary

1 Evidence is given from case study of reticulo-endothelial disease supporting the concept that monocytic leukemia is one form of reticulo-endotheliosis

2 On the basis of varied types of cell reactions seen in monocytic leukemia it is suggested that all forms of leukemia may be hematologic varieties of reticulo endotheliosis

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THE POSSIBILITY OF PRECIPITATING THE LEUKEMIC STATE BY EMOTIONAL FACTORS

By F R MILLER M D AND H W JONES, M D

LEUKEMIA in the human individual may develop without any known pre cursor but frequently it occurs following a precipitating incident or set of incidents It is apparent from a review of the literature that various physical chemical and possibly infectious agents are involved in the inception of some of these diseases [1] Exposure to irradiation from x ray or radium contact with or exposure to benzol or its derivatives physical trauma and the use of arsenical and sulphonamide drugs are some of such agents Infections which seem to have played a part in precipitating these diseases are tuberculosis syphilis and pneumonia The relation of infection in this regard is not as well established as that of the physical and chemical agents Exposure to benzol and exposure to irradiation from x ray or radium are more clearly related to the precipitation of the leukemic state than is exposure to other agents

No one believes that these agents cause leukemia but it is possible that each may act to upset the normal balance of blood formation so that leukemia results

Results of experimental work [3,4] point to the normal control of blood forma tion by hormonal as well as dietary factors If leukemia is precipitated by these chemical and physical agents this probably is brought about by changing the hormonal balance In those cases of leukemia in which such agents are not un covered in the pre leukemic history it is possible that emotional factors may have acted in a similar role in precipitating the leukemic state

For many years the influence of emotional reactions on physiologic processes has been well known but only within comparatively recent times have studies reveal d the significance of emotional factors in precipitating or aggravating a number of disease processes such as peptic ulcer asthma mucous colitis etc As the etiologic importance of these factors has been demonstrated a much better understanding of these illnesses has been brought about Milkorat[5] and his co workers showed that leukocytosis with a normal differential appeared to be intimately related to the psychopathologic emotion They explained the elevation of the total white cell count on the basis of a redistribution of the white cells from the organ reservoirs It is well known that there is an intimate interrelation ship between emotional factors the autonomic nervous system and the endocrine glands These findings therefore have led us to believe that the frequent occurrence of emotional difficulties in patients with leukemia may be more than a coincidental finding Often these patients volunteered a great deal of material concerning their own psychologic difficulties Therefore we have endeavored in a few cases of leukemia to determine the emotional background and in this paper we wish to present and discuss the emotional histories of 6 cases prior to the development of leukemia

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Case Reports

Case 1 K R was a 26 year old white man who entered Lakeside Hospital Cleveland March 1939 His boyhood was normal up to the time he entered junior high school At this time in 1929 his father suffered severe financial losses He stated I began to worry more than my father He graduated from high school in 1931 and by this time his father had lost everything except his home At night he would lie awake trying to figure out what he and his father could do to stave off financial disaster He went to college and worked part time to pay his tuition His college work was of very poor quality the first two years and he had a very fatalistic attitude concerning it It was difficult for him to work because of worry over finances—not only his own but his father s His work improved in the third and fourth years because he realized that he would not graduate unless he applied himself It was necessary to go to school an extra trimester in order to have sufficient credits to graduate He met his wife in his second year in college 1933 but was not married until 1938 No sexual intercourse occurred during this time but the patient did develop an enlarged prostate that required massage The long courtship wa a definite strain and he worried concerning how he could support a wife In March 1937 he developed fullness in the left upper quadrant of his abdomen In August 1938 he had considerable belching and nausea and vomiting A physician at this time told him he had a nervous stomach

When the family had heavy financial losses in 1929 the patient s mother became emotionally upset and worried a great deal She confided in him rather than in his father and this gave ri e to emotional turmoil in him During the last year of college he was greatly depressed and cried frequently There were no death wishes He worried and became very tense the summer of 1938 just before being married he could not eat and gagged after meal and at times he vomited He felt that he was close to a nervous breakdown just before he was married

He seemed to be an extremely tense worrisome individual who was rather sensitive and proud and somewhat aggres ive He resented being dependent on anyone Leukemia was apparent four months after his marriage He lived one and one half years following the diagnosis of leukemia Necropsy was performed in the Jefferson Hospital and the anatomic diagnosis substantiated the diagnosis of chronic myeloid leukemia

Case 2 K H was a 57 year old white woman of Russian birth She entered Jefferson Hospital Philadelphia late in the summer of 1939 A diagnosis of chronic myeloid leukemia had been made five years before The following history was obtained from her si ter The patient had been a timid introspective child she had cared more for book and studies than for social gathering and was a good student After she had finished high school in Russia she met and fell in love with a young man His family disappro ed of their love affair and he was not allowed to marry him This was a great disappointment to her and even up to the latter part of her life she mentioned him and stated that her life might have been different had she been permitted to marry him

While she was still in high school he tarted to study music When she was 22 another man fell in love with her and after much per i tence he persuaded her to marry him She was fond of him but probably ne er really l ed him He was domineering and egocentric and her life with him was probably never completely happy Immediately after their marriage he had difficulty in earning enough to provide for the two of them o that he helped by giving music lessons Early in their married life she had a nervous breakdo n and could not speak for a time She also had several amnesic episodes Soon after these occurred the yo ng couple went home to live ith her parents ith whom they resided for two or three years During thi time a on was born Their fortunes improved and they had their own home and no financial difficulties until the Ru ian re olution in 191 Her father died in 1915 and this was a great shock to her as was her mother death in 1917 Following the revolution her little family found it necessary to leave R issia and ent to Germany Difficulties in Germany in the early tw enties brought them to thi country F nally her marriage ended in divor e Her l fe had been one disappointment after another

The diagnosis of leukemia wa m de h e ye r prior to her entry into Jefferson Hospital She died in Octob r 1939 and the necrop y find ngs ve e th e of chroni myeloid leukemia

Ca e 3 S F was a 39 year old hite man He entered Lakeside H spital Cleveland in the winter of 1939 A psychiatric int r ie as obtained Ja u ry 19 1939 Thi man wa a chron c worrier He had been

brought up on a farm but at the age of 21 he was re tless and ambitious to go into business He married at 20 and his wife was never well At 22 he worried over financial losses incurred in a filling station which he owned His wife died four years after their marriage while giving birth to a child He was worried and grief stricken but he worked hard and did fairly well in business saving about $20 000 up to 1929 From 1930 through 1933 he was worried about finances most of the time He lo t his business spent his entire savings and then went back to work for his father on a fruit farm There he made no money was depres ed and later fought with his father and left the farm discouraged and unhappy Throughout this entire time he was physically well In 1934 he again tried to go into business determined to make good and throughout 1934-7 he was not so easily worried and his finances were a little better managed In 1937 he had his teeth extracted and he bled considerably and this again worried him He also tired easily at this time and had no pep In 1938 he used up his savings again was unable to work well and every thing then seemed to go from bad to worse He was afraid and seemed to have lost his nerve and he cried easily Late in 1938 the diagnosis of chron c myeloid leukemia was made This patient died at home May 1940 A necropsy was not obtained

Case 4 G P was a 46 year old man who was first seen in the Jefferson Hospital Philadelphia clinic the winter of 1940 He had had chronic myeloid leukemia for six years and had been treated in several other clinics A psychiatric interview was held in October 1940 He was of German American extraction His school work had been of good quality but he went to work immediately follo ing graduation from high school At about 25 years of age he went to work for a rubber company and a year later was married but almo t immediately after ards he was transferred from New Jersey to a plant in Canada Here he was pla ed in complete charge of con truction production and sales of a new branch of his company His ife however remained in the United States the first year Almost the entire burden of responsibility of the new plant was carried by him He had few friends and almost no one to whom he could confide his d fficulties He was under considerable tension and felt that the re ponsibility was very great At this time he began to have queer sensations in his stomach and his digestion was poor He became easily fatig ed and had great difficulty in sleeping A little later he began to have attacks of vomiting and severe headaches These symptoms lasted for several years and ere pronounced whenever the pres sure of business was heaviest

His first child as born when he was 34 At the age of 37 or two years before leukemia was apparent his branch of the company lost $ o 000 during the year This was an added source of anxiety to him and he took the responsibility for the loss His second child was born four months before the diagnosis of leukemia was made At this time he was overworked and worried because of the company finances as well as his own He developed an infection of his neck and during the care of this leukemia was dis covered

Anxiety and worry concerning his family and company were not lessened with the diagnosis of leukemia but he became philosophic He said that he bel e ed there were spontaneous remissions and that he would have one He read much concerning leukemia and he tried to help in caring for himself

He stated that he had had definite mood sw ngs all his l fe some weeks he felt on top of the world other eeks he was down in the dumps and every little job looked twice as big as it ought to His leukemia was discovered in December 1933 and continued through March 1941 or over seven years He died in Memorial Hospital New York City March 1941 and a necropsy re ealed typical findings of chronic myeloid leukemia

Case 5 P H was a 53 year old white man He entered Lakeside Ho pital Cleveland in the inter of 1939 where a diagnosis of chron c myeloid leukemia was made In February 1939 a psychiatric inter view was obtained The patient had worried over small things his entire life In 1918 he orried himself sick about everything He had been a ca etaker on n estate and he had worr ed about his duties he did not belie e he was doing well and e eryth g seemed to go wrong He became depressed and could not sleep and he lost his appetite The estate as then sold and he became caretaker on another The owner comm tted s icide but before this h ppened he had felt better and was doing rather well The su cide was a shock to him Following this episode he had worked on yet another estate for a period of eight years While working on this estate he w s worried and ens tive to criticism During this time his father died and he became depressed He worked night and day but seemed to be in a rut He again changed his position and became even more wo ried At this time he believed someone was poisoning the

pheasants he was raising and thought that this was being done to change the control of the estate This led to a fight with another man on the estate

In August 1938 he became extremely depressed and wanted to die He thought people were making fun of him and believed that someone had it in for him and was plotting against him He thought that the world was wrong Then he lost interest in everything and could neither eat nor sleep He lost his self confidence and was always afraid he would make mistakes Although he had death wishes he had no suicidal ideas He cried a great deal and he tried to be alone and would walk the streets His depression grew worse until in November 1938 he was physically sick At this time he had pains in both hips and numbness and tingling from knees to feet He had no vitality or energy and felt a pressure on top of his head and pain in the back of his neck He thought he was going to die and made a will and just waited In February 1939 it was found that his spleen was enlarged and that his leukocyte count was elevated The patient died in Lakeside Hospital August 1941 The necropsy findings were typical of chronic myeloid leukemia

Case 6 M A a 40 year old white woman entered Jefferson Hospital Philadelphia in December 1946 A psychiatric interview was held in January 1947 The patient appeared pale underweight and considerably older than the age of 40 She wept when she talked about her husband She is married to a miner and has nine children Her husband is an alcoholic who has been cruel and a poor provider After twenty years of marital difficulty she finally forced her husband to leave their home in October 1945 At present she has $93 a month from Mother s Relief and a few dollars irregularly from her husband to care for her nine children

She was one of four children Her father was an alcoholic and he was unstable She stopped school at the age of 14—the 6th grade—because she had to work and help support the family Despite her difficult life there were few somatic complaints until two years ago when she began to be easily fatigued and lost weight It was at this time that the difficulty with her husband became acute

She is a Catholic and very religious The impression of the interviewer was life long insecurity related to difficult environment and a lack of affection as a child Her main defense was compliance religion patience and acceptance It is difficult to evaluate the exact relationship between her emotional difficulties and her present disease but it is apparent that the emotional difficulties have played a part in the development of the organic disease

It was found in October 1946 that her spleen was enlarged and the leukocyte count was elevated She is now being given treatment for chronic myeloid leukemia

Discussion

In this short series only cases of chronic myeloid leukemia have been included Psychiatric interviews however have been held with 3 patients with chronic lymphoid leukemia and only one of these has given much evidence of emotional difficulties in the background One patient with chronic eosinophilic leukemia was also interviewed and it was found that following an accident in the plant in which he worked he frequently had somatic symptoms when he was in the room in which the accident had occurred Otherwise there was little in his pre leukemic history which might have acted to precipitate the disease

Psychiatric interviews have not been held with patients with acute leukemia nor have we examined the emotional background of either patients or parents of any of the childhood leukemias

Clinical data of all types have been left out of this report because each case represented a typical picture of chronic myeloid leukemia In only one of these patients Case 4 was there any evidence in the pre leukemic history of chemical or physical agents which might have precipitated the leukemia This man worked in a rubber plant but he was not exposed to chemical agents

Anxiety depression and chronic worry affected 4 of the 6 patients of whom

case reports are given Each of these 4 had conversion symptoms such as nausea vomiting and loss of appetite One had pain in his hips and a feeling of pressure at the top of his head Three of these 4 had loss of appetite and sleeplessness Three of these 4 cried easily so that it might be said that each of the 4 was somewhat emotionally unstable prior to the development of leukemia One of the other 2 had had emotional strain as a girl and later hysteria difficulties and disappointments because she married a man she did not wholly love The sixth had lead a life of misery as a child and for twenty married years It may not be entirely convincing that the material in such histories has precipitated the leukemic state Heuper[1] has stated that the leukemic state has not been precipitated by emotional or psychic trauma These histories however run somewhat contra to this statement

Recently it has been shown that in the past forty five years there has been a marked increase in the incidence of leukemia [5] Drugs industrial hazards irradiation from x ray and atomic sources and the increased hazard of physical trauma of our age may have brought about this increase The increased tempo of life in the past forty five years with its increased sources of anxiety worry and emotional *stress and strain may also have aided in increasing the number of deaths from* leukemia

It seems to us that all cases of leukemia in adults should be studied for any and all factors which may have played a part in the precipitation of the disease and such a study should include emotional factors Against a statistical study of emotional factors in all cases there should be plotted a particular study of those cases in which other factors have been ruled out

Summary

The emotional background of 6 cases of chronic myeloid leukemia are reviewed after psychiatric interviews In 4 emotional instability was of long standing and in these 4 chronic worry and anxiety seemed to be an integral part of the pre leukemic history One of the other 2 suffered emotional trauma early in life and one *had lived a life of misery as a child and an adult* Further study of emotional factors in relation to pre leukemic histories should be made

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THE PRESENT POSITION IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA

By A PINEY M D MRCP

IT IS not proposed to devote this article to a detailed description of the various methods of treating chronic myeloid leukemia or to a statistical assessment of the results The aim is rather to consider the theoretic bases on which the different forms of therapy have been devised, while incidentally, indicating whether the observed results are such as to support the hypotheses

That the problem of the etiology of the leukemias is almost as obscure as it was a century ago when Hughes Bennett and Virchow independently of one another published the first accounts of the disease is indisputable And this assertion is not invalidated by the discovery of the transmissible leukemias of lower animals which has in fact complicated rather than clarified the problem of the disease as it is observed in man But in spite of the distressing lacuna in our knowledge research into the treatment of the chronic forms of leukemia has been pursued in a manner which has on the whole been scientific rather than purely empiric And this has been possible because we are less abysmally ignorant of the underlying pathologic and cytogenetic factors than we are of the causative ones

For instance it is patent that in the leukemias there is gross overgrowth of hemopoietic tissue which in the case of our present subject of discussion—chronic myeloid leukemia—is the bone marrow And at this point it is important to realize that the intense proliferation of white cells in the marrow is not to be considered as an example of hyperplasia because that concept includes that of increase in the number of normal cells in normal arrangement with as a result increased but essentially normal functional ability Thus the extremely active cellular marrow found in many infections is an example of hyperplasia whereas the more or less disorderly proliferation in myeloid leukemia is to be considered as pathologic overgrowth And this is true whether the malady be included with the neoplastic ones or not

Now in normal postnatal life the mature granulocytes found in the circulation arise from mitotic division of granular myelocytes That is to say the polymorphonuclear leukocytes with their specific granulation arise from simpler cells which do however already possess characteristic granules in their cytoplasm Less mature cells—myeloblasts—are not involved in the process forming as it were a reserve of stem cells which are not normally called into action and even in severe infections the marrow contains few myeloblasts whereas myelocytes are very numerous and show signs of active proliferation In fact it is probably true that extension of the genealogic tree back to the myeloblast stage results in the formation of abnormal granulocytes In other words there is reason to suppose that the abnormal white cells which are so prominent a feature of the blood and marrow in chronic myeloid leukemia are produced from maturation of myelo

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blasts and this view is consistent with the generally accepted statement that the outlook is the worse the greater the proportion of myeloblasts in the marrow

Deterioration that is to say a tendency towards acuteness in chronic myeloid leukemia is detectable in the marrow earlier than in the blood because there is great overgrowth of myeloblasts (at the expense of myelocytes) *before* there is any noteworthy change from the chronic type of blood picture It is therefore reasonable to assume that conversion of chronic into acute leukemia comes about by a peculiar process of dedifferentiation

First there is increase in the number of myeloblasts in the marrow although some or many of these cells have the power of maturing into more or less normal myelocytes which in turn may give rise to polymorphonuclears or they may themselves emerge into the circulation Secondly there is still further deterioration of the functional activity of the myeloblasts which lose their power of becoming differentiated into myelocytes and this represents the stage of complete and irreversible conversion of chronic myeloid leukemia into the acute form Of course intermediate phases are well known as witness the presence of ill formed myelocytes in the peripheral blood

The brief discussion above will serve as a preface to the more distinctly therapeutic problems which confront us while later a rather more recondite consideration of the cytologic factors will be required as an introduction to the most modern methods of treatment

Probably the oldest method of treating chronic myeloid leukemia is by administration of arsenic and this is especially interesting as an example of more or less successful empiricism because the mode of action of the drug in blood dyscrasias is still obscure Now of two facts there can be no dispute first arsenic properly exhibited can bring about clinical and hematologic remission in many cases and secondly that this is brought about by decreasing the activity of myeloblasts in reproducing themselves and in giving rise to myelocytes In other words arsenic at least for a time is able to cause leukocytopoiesis to proceed along more or less normal lines while at the same time decreasing the gross overactivity of myelocytes in dividing and in differentiating into polymorphonuclears

Physicians of a past generation who like us were not averse to hiding ignorance under a cloak of words valued arsenic for what was known as its alterative action And it is indisputable that this term is admirably descriptive of the effect of arsenic in chronic myeloid leukemia This was equally well shown before the introduction of liver treatment in pernicious anemia by the effects on the erythrocyte picture

There are many points of practical importance in connection with arsenical medication as Forkner and Scott emphasized in 1931 when the drug which had almost fallen into disuse in the treatment of leukemia was given a new lease of usefulness

A few of the advantages may be mentioned here Thus arsenic is cheap and easily available and if given in the early stages of chronic myeloid leukemia it usually produces a good remission which can sometimes be maintained for months or even years by continuing to administer maintenance doses This second feature is a great

advantage over irradiation which has of course to be interrupted when the blood picture is more or less normal as, otherwise, aplasia of the marrow may ensue

Not only does arsenic cause a reversion of the leukocyte picture to a more normal composition but it produces amelioration of the anemia But it is debatable whether this is the result of a direct action of erythropoiesis whether it is due to relief of pressure on the red stem cells or whether both (and perhaps other factors also) play a part

This is not the place to describe the minutiae of therapeutics but it is well to point out that when arsenic will no longer maintain the patient in a state of remission irradiation may still do so However it is essential to recollect that exposure to therapeutic irradiation shortly after a prolonged course of arsenic is likely to cause more severe reactions than would a similar dose of x rays in a patient who has not had the drug This is alleged to be due to secondary radiation from the arsenic stored in the tissues but whatever the explanation a history of having taken arsenic for months before starting radiation treatment indicates the need for small and experimental dosage of x rays

Conversely arsenic may be effective in some cases which have become resistant to x rays still inducing a remission when irradiation will no longer do so This is of course true only when the blood and marrow are still characteristic of the chronic form of the disease but if the failure of x rays is due to conversion into the acute (myeloblastic) state no known treatment will bring about a remission

Another method of medicinal treatment which like arsenic has to some extent fallen into undeserved disuse is benzol which also has the advantage of being given by mouth while the dangers which have been attributed to its administration are entirely the result of gross overdosage

Benzol is a well known marrow poison which has caused a good deal of trouble in various industrial processes for this reason It appears to damage the platelets the granulocytes and the red corpuscles in that order affecting the parent cells and so reducing the mature forms in the blood

It was this knowledge of the action of benzol which led to its use in treating leukemia but there is no doubt that its effects are not entirely due to its myelotoxic action If given in suitable doses the leukocyte count falls as a result of decrease in the number of immature and abnormal white cells while the red count rises and the changes in the marrow are of the same type viz decrease of myeloblasts and a more normal activity of the myelocytes In fact a new investigation of benzol is overdue because it has now been established that prolonged exposure of healthy persons to small doses of benzol greatly increases the likelihood of the development of chronic myeloid leukemia

If benzol is given when the leukocyte count is very high it is best exhibited in capsules with olive oil each containing 0 5 Gm of benzol The course is started with four capsules daily and if there is no nausea is rapidly increased to a maximum of eight or ten daily This dose is continued until the leukocytes have fallen to about 50 000 per cu mm when the dose is gradually reduced until the white cell count is about 15 000 per cu mm Then it is often possible to find a suitable maintenance dose usually in the region of 0 5 Gm two or three times a week

Again like arsenic benzol is still effective when radiation has become ineffective but unlike arsenic a preliminary course of benzol is not liable to cause trouble during subsequent radiation treatment

Benzol acts as an essentially destructive agent which has a rather more powerful effect on immature cells than on mature ones hence the beneficial results in chronic myeloid leukemia Unlike radioactivity and urethane its effect on dividing cells is no greater than on resting ones so that it cannot be regarded as affecting the essential abnormality which is the cytologic basis of the disease

For many years the usual method of treating chronic myeloid leukemia has been with x rays and as the results obtained are rapid and spectacular arsenic and benzol have fallen into a poor second place As already indicated the dislike of these two drugs is an ill founded one

Deep x ray therapy can rapidly bring about a remission in the great majority of cases of chronic myeloid leukemia but that is no reason for assuming that the radiologist is the right person to have charge in such cases The disease lies in the province of the physician who is in a much better position than is the specialist radiologist to determine the most suitable therapy and to assess the results in individual cases

This is not a denial of the right of the radiologist to determine which particular technic of irradiation is to be employed but despite quite acrimonious divergences of opinion between different schools it can truthfully be said that the results obtained by the different methods are all approximately the same The length of the remission which is brought about is no longer when one radiologic technic rather than another is employed and the development of radioresistance is not accelerated or postponed by any particular dosage or by exposures of different areas

It is probably no exaggeration to say that the development of therapeutic irradiation in cases of chronic myeloid leukemia has never been exploited as fully as it might have been Most physicians have been satisfied with the remission brought about by x ray treatment and have kept careful watch for the earliest hematologic signs of relapse and when these have appeared have returned the patient to the radiologist for further treatment

The mild contempt with which arsenic has been regarded and the fear of benzol as a possible cause of aplastic anemia have prevented combined treatment from being used as extensively as might have been expected from our empiric knowledge of therapeutics In part of course this failure has been due to the habit of handing cases of chronic myeloid leukemia to the radiologist

That the length of a remission which has been induced by x rays can be prolonged by administration of maintenance doses of arsenic or benzol is indisputable but there are no published records of individual cases which have been dealt with in this way More and more attention has been given to irradiation and less and less to medicinal treatment Thus sodium phosphate made radioactive by the cyclotron has been hailed as a great advance in treatment whereas in fact its main advantage is that it can be administered orally while the results have been

much the same as those of other methods of exposing the leukemic cells to the destructive action of rays

Even the most enthusiastic advocate of radiation treatment has not it may be assumed, ever supposed that the method, however modified it might be in the future, would result in cure of leukemia Some workers have sought for a hypothetic virus others for a toxin, and yet others for indications of a chemical or an endocrine factor and it is probably correct to say that more and more adherents to the chemical (or constitutional) theory are won annually although of course acceptance of this view does not exclude leukemia from the neoplastic class

Only a very few indications of the evidence can be given here but they may suffice to stimulate further work while they form a more or less rational basis for the further discussion of medicinal treatment First there is the well known fact that the incidence of leukemia may be familial although admittedly this is far from common Nevertheless in the case of a disease so relatively rare the existence of such cases suggests that some intrinsic factor is of etiologic importance and such a view is supported by the distinctly greater frequency of the chronic myeloid form in Jews Secondly the fact that long continued exposure to small concentrations of benzol (and perhaps of x rays) increases the incidence of chronic myeloid leukemia seems to demonstrate that a chemical change underlies the abnormal proliferation which characterizes the disease

But perhaps the best evidence indirect though it is may be found in certain more academic observations Thus the cytologic changes which are accepted as being indicative of malignancy are present in more or less well defined form in leukemia The most conspicuous of these changes are hyperchromatism which is due to increase in the size of the chromosomes enlargement of the nucleolus variation in the number of chromosomes defects in the spindle during mitosis and increased variability in the size of the cells and the nuclei Obviously the question whether there is any casual sequence among these cell abnormalities demands answer

An inadequate but I hope accurate review of the fundamental work of Caspersson Darlington Claude and Thomas will give some indication of the present position of the problem Thus it is known that nucleic acid and nucleoproteins play an outstanding part in cellular activities Ribose nucleic acid is produced by the heterochromatic regions of the chromosomes and is found in the nucleoli and in the cytoplasm It is closely associated with the synthesis of the self perpetuating proteins in the cell body Desoxyribose nucleic acid which is produced during the prophase of mitosis becomes attached to the chromosomes and is responsible for their reproduction While this is happening the nucleolus with its store of ribose nucleic acid dissolves and disappears Then when the chromosomes have divided and have reformed as daughter nuclei their charge of desoxyribose nucleic acid is given up and is reconstituted as ribose nucleic acid in the freshly formed nucleoli

In normal cells the two nucleic acids are so balanced that chromosome reproduction and cytoplasmic synthesis are balanced probably by the regulating action of heterochromatin If this be so increase of heterochromatin will lead to excessive nuclear synthesis and therefore to an increased rate of nuclear division

Endless and rather fruitless speculation along these lines is possible but even without departing far from solid experimental observations something of importance can be inferred For instance Beadle showed that mutation in a single nuclear gene can induce polymitosis but the observations briefly discussed above indicate that mutation in cytoplasmic elements may also cause great change of cellular characters Then again the existence of self perpetuating elements in the cytoplasm (the plasmagenes of Darlington) throws some light on the characters of viruses because both plasmagenes and viruses depend for their continuance on a chemical equilibrium and Potter alleged that the cancer virus is almost identical with an enzyme X which is a complex of respiratory enzymes of the nature of a ribonucleoprotein

The chemical and the virus theories of the origin of malignant conditions among which leukemia must be included (at least on cytologic grounds) are thus found to be almost if not entirely unified

These observations and similar reflections together with further experimental work form the basis of the urethane treatment of leukemia introduced by Haddow and Sexton and investigated by Paterson Haddow and others And one point that emerges very clearly is that nucleic acids are of outstanding significance in the leukemias (and probably in all forms of malignancy) This is strikingly shown by the action of colchicine which was first employed in medicine for its action in gout (a malady in which the metabolism of purins is upset) was then observed to act on the bone marrow later was discovered to have a remarkable effect on mitosis (for instance in inducing polymitosis in wheat) and is now known to have some effect in leukemias And when the fairly common concomitance of gout and chronic myeloid leukemia is recollected the skein of evidence incriminating the purins although still unravelled seems to be fairly complete

During experiments on the growth inhibiting effects of urethane on animal tumors Haddow and Sexton observed striking changes in the cells of the Walker rat carcinoma and a fall of the leukocyte count in some cases and as there is little evidence that urethane is liable to cause aplasia of the marrow it is preferable to benzol while being more efficacious than arsenic This suggested the trial of the drug in leukemias and it is not too much to assert that further therapeutic application of this substance has shown that it is probably the most satisfactory treatment for chronic meyloid leukemia now available

Urethane remains effective when x rays have failed but seems to be the method of treatment indicated from the outset in most cases But it is not to be supposed that the drug is in fact a cure for the disease

The composition of the leukocyte picture approaches normal the red cells and hemoglobin rise and the spleen retires behind the costal margin And it appears that these results depend upon the action of urethane at the prophase of mitosis of the least differentiated stem-cells of the blood There is in fact a redistribution of the nucleic acids as shown by the Feulgen reaction The way is thus open for the micro-chemist to find a new weapon for the even more fundamental treatment of the leukemias but already urethane can often produce a prolonged period of clinically perfect remission

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THE USE OF URETHANE (ETHYL CARBAMATE) IN THE TREATMENT OF LEUKEMIA

A Preliminary Report

By Charles H Watkins M D Talbert Cooper M D
and Herbert Z Giffin M D

HADDOW and Sexton[1] noted a coincidental depression of the leukocyte count during the course of observations on the effect of various forms of urethane on the growth of experimentally produced tumors in the rat As a result the various forms of urethane principally ethyl carbamate were subsequently utilized in the treatment of leukemias and other types of malignant disease by Paterson Thomas Haddow and Watkinson [2] Encouraging results followed the administration of urethane in the majority of 13 cases of myelogenous leukemia and to a lesser extent in 9 cases of lymphatic leukemia The most favorable response consisted of a reduction of the total leukocyte count to normal levels production of a more nearly normal differential pattern elevation of hemoglobin levels and diminution in the size of enlarged lymph nodes or spleen These effects were sustained for variable periods the longest period of observation recorded being eleven months Symptoms of gastrointestinal irritation of mild to moderate degree rather commonly followed administration of the drug Aplastic anemia developed in 2 cases in one of which there was a fatal termination In general the results obtained were regarded as much like those following the use of the standard method of irradiation therapy

The mode of action of urethane remains obscure Kirschbaum and Lu[3] observed that the administration of a single anesthetic dose of urethane to mice with myelogenous leukemia resulted in a drop within twenty four hours in the total leukocyte count and in the appearance of many mature cells in the bone marrow The number of mitotic figures in the myeloid cells of the marrow was decreased and it was suggested that maturation may have been secondary to inhibition of mitosis in blast cells Johnson[4] observed that urethane exerts an antisulfanilamide effect similar to that of para aminobenzoic acid in work with certain strains of luminous bacteria This would suggest that urethane s growth inhibiting property may result from interference with utilization in cellular metabolism of some natural amine

At the Mayo Clinic clinical experience with urethane (ethyl carbamate) in the treatment of leukemic states now covers a period of approximately eight months While the ultimate evaluation of this substance as a means of treatment for the leukemias obviously remains to be determined in the future certain current observations may be of interest to those concerned in the management of these perplexing conditions

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Results of Treatment

Chronic myelogenous leukemia In 14 cases urethane is now being used as the sole means of treatment The patients in these cases have been under treatment for periods ranging from seven weeks to eight months In all instances the response to treatment thus far has been regarded as satisfactory and no supplementary measures have been required There has been a consistent reduction in the total leukocyte count (table 1) accompanied by a parallel reduction in the degree of myeloid immaturity apparent in differential analysis of smears of the peripheral blood and sternal bone marrow The erythrocyte count and hemoglobin values have either shown a significant increase or have remained virtually unchanged In no instance has anemia or clinically significant thrombocytopenia developed during

Table 1 —*Early Effect of Treatment of Chronic Myelogenous Leukemia with Urethane*

Urethane Gm	Leukocytes per cubic millimeter of blood	
	Before treatment	Six weeks (average) after treatment
65	124 000	23 500
95	234 000	93 600
105	155 000	18 600
68	163 000	21 100
87	145 000	25 600
110	307 000	26 000
91	147 000	46 000
75	260 000	13 000
40	137 000	9 590
52	130 000	23 400
168	210 000	84 000
21	164 000	8 300
85	118 000	14 750
72	117 000	42 100

the course of treatment Splenomegaly has been consistently and rapidly diminished in degree although in no case has a previously palpable spleen disappeared entirely The degree of response apparently was not affected by the initial level of the total leukocyte count the duration of the illness or the amount of previous irradiation therapy

A satisfactory response to treatment was achieved in three to ten weeks (average six weeks) following the administration of 21 to 168 Gm of urethane (average 80 Gm) The drug was usually administered in a dosage of 1 Gm three times a day at the outset This amount was reduced to 1 to 2 Gm daily as the leukocyte count descended The primary fall in the leukocyte count usually occurred seven to fourteen days after the institution of therapy and was often preceded by a transient elevation However in 2 cases twenty one to twenty-eight days of treatment was required to produce the initial depression of the leukocyte count

The dosage necessary to maintain the leukocyte count at relatively normal

levels (less than 20 000 per cu mm of blood) has been highly variable although for the 2 patients under observation for the longest periods 0 5 to 1 0 Gm daily has been found adequate Weekly leukocyte counts are necessary in determining the long term needs in the individual case the amount of urethane administered being increased or reduced accordingly An effort has been made to maintain the total leukocyte count at 20 000 or less per cu mm and the drug has been temporarily discontinued when the total leukocyte count has been less than 10 000 per cu mm No cases of chronic leukop nic myelogenous leukemia were included in this series

On cessation of therapy recurrence of leukocytosis with myeloid immaturity invariably occurred after variable periods of time

Mildly to moderately severe symptoms of gastrointestinal irritation occurred in approximately one third of the cases but disappeared with continued administration of the drug although a reduction in dosage was sometimes necessary A direct relationship between the size of the daily dose and the frequency of gastrointestinal complaints was soon established and in recent months an amount in excess of 3 Gm daily has rarely been prescribed No other toxic manifestations attributable to the drug were observed in this series

Acute myelogenous leukemia Urethane was administered in 2 cases for periods of ten to fourteen days with no significant change in the clinical course although there was some decrease in the degree of myeloid immaturity in smears of the peripheral blood and sternal marrow

Chronic lymphatic leukemia The drug was administered in apparently insufficient amounts in 2 cases There was no appreciable effect on the total leukocyte count

Acute lymphatic leukemia Urethane has been administered for periods of one to three weeks in 8 cases In three instances there was a precipitous fall in the total leukocyte count but in none was the general clinical course materially influenced

Acute monocytic leukemia In 2 cases treatment of one to three weeks duration produced no measurable hematologic or clinical change

Comment

Our experience would indicate that urethane can be expected to produce a temporary hematologic remission in cases of chronic myelogenous leukemia This remission is similar in superficial characteristics to that observed after irradiation therapy There is at the present time no indication that urethane offers more than other agents which are used palliatively in this disease However by virtue of its convenience of administration and the possible advantage of controlled continuous action urethane may be found preferable to other methods of treatment in use at the present time

We have had insufficient experience with the use of urethane in the treatment of chronic lymphatic leukemia to warrant an opinion as to its efficacy However the reported experiences of Paterson and co workers[2] would seem to justify continued use of the substance in such cases

While like irradiation therapy urethane may produce a rapid decrease in the number of immature leukocytes circulating in the blood in some cases of acute

myelogenous and lymphatic leukemia, there is no evidence to suggest that the usual course of these conditions has been beneficially influenced

Despite the absence of serious toxic effects encountered in this series to date the potential production of aplastic anemia and other complications[5] by this substance must be kept in mind

SUMMARY

Limited experience with the use of urethane in the treatment of leukemia indicates that this substance presents a considerable promise as a palliative agent in chronic myelogenous leukemia It has no apparent value in the treatment of acute leukemia

Further extensive observation will be necessary to provide a true measure of the clinical usefulness of this preparation

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URETHANE THERAPY IN LEUKEMIA

By Adolph J Creskoff M D Thomas Fitz Hugh Jr M D and John W Frost * M D

THE purpose of this paper is to report the results of urethane therapy of the leukemias based on a study of 24 patients

In April 1946 Haddow and Sexton[1] described the influence of various carbamic esters on experimental rat cancers Ethyl carbamate (urethane) yielded the best results It alone produced inhibition of tumor growth and fibrous replacement of the cancerous rat tissue For this reason and since urethane is relatively nontoxic for humans and is easily obtainable the experiments were transferred to human subjects with carcinoma of the breast and other malignancies The results were generally disappointing It was however noted that a few of these patients developed leukopenia while taking urethane This observation motivated in 1943, the first clinical trial of the effects of urethane in leukemia and allied disorders

In May 1946 Paterson Haddow, Thomas and Watkinson[2] reported the results of urethane treatment of 32 leukemic patients The drug was administered orally in an average dose of 3 to 4 grams daily The drug proved effective in approximately one third of the patients producing in this favorable group reduction in size of enlarged spleens and of lymph nodes and causing reversion of the blood picture to more normal values These workers found the urethane effect to be approximately equal in value to that of standard deep x ray therapy in a control series of similar cases Of their 32 urethane treated patients 19 had myelogenous leukemia and 8 of these were benefited Clinical and hematologic remissions were maintained for periods of 2 to 6 months Of 13 patients with lymphatic leukemia 2 responded similarly The remaining 22 were either partially improved could not tolerate the drug or died during treatment

Toxic side effects observed by the British workers included nausea drowsiness anorexia diarrhea and suggestive evidence of marrow hypoplasia

On the basis of these results parallel observations were started at the Hospital of The University of Pennsylvania in June 1946 At the time of this writing (September 1947) we have treated a total of 27 patients suffering from leukemia Three of these are too recently treated for accurate evaluation Our report is based on the remaining 24

Dosage and methods of administration The usual dose of urethane was 4 grams (range 2 to 6 Gm) daily given in solution orally A mixture similar to that used by the British group was employed in most of the patients

Urethane	30
Syrup of orange	50
Chloroform water to	300

(each 5 cc contains 0 5 grams urethane)

From the Hematological Section of the Medical Clinic Hospital of the University of Pennsylvania Philadelphia

This work has been aided by a grant from the Eli Lilly Company

* Eli Lilly Fellow in Hematology

This mixture was administered (1 to 4 teaspoonsful) after each meal in a small amount of some flavored carbonated beverage or fruit juice. In a smaller group of patients the drug was administered in 0.5 Gm. gelatin capsules. This method of administration proved to be impracticable because of the hydroscopic nature of the urethane. Rectal suppositories (each 0.5 Gm. urethane in cocoa butter) were tried in 2 patients who developed nausea from oral medication. Rectal irritation occurred within a few days requiring cessation of this method of administration.

In one patient (Case 4) with a large leukemic tumor, 50 per cent urethane ointment was employed locally in conjunction with oral urethane medication. The ointment was prepared by melting urethane crystals and incorporating the solute in aquaphor. The leukemic tumor disappeared under this therapy although the patient died subsequently.

Urethane intravenously has been our most recent mode of administration. Ampoules* containing 1 or 2 Gm. urethane in 20 cc. of normal saline solution or distilled water were mixed with 200 cc. of normal saline and the resulting mixture was administered in 15 to 30 minutes intravenously without untoward reactions.

Clinical material. The results in 24 leukemic patients are reported. Table 1 indicates the distribution of types of leukemia.

TABLE 1

Acute Leukemias		10
Myelogenous		
Lymphatic		
Monocytic	1	
Chronic Myelogenous Leukemia		7
Chronic Lymphatic Leukemia		7

RESULTS OF URETHANE THERAPY

Chronic Myelogenous Leukemia

Our results in 7 cases of chronic myelogenous leukemia are summarized in table 2. It is to be noted that in 4 cases urethane therapy was started during terminal stages of the disease after resistance† to x-ray therapy had been established. Two cases are regarded as showing satisfactory clinical and hematologic remissions after urethane therapy. Case 4 is of special interest because of a successful remission maintained despite the complication of pregnancy which is now in its seventh month and is proceeding uneventfully.

CASE REPORTS

Case 4. This white female, age 30, first noted weakness, nausea, and abdominal enlargement during the latter months of 1945. When first examined on December 12, 1946, she exhibited massive splenomegaly (19 cm. below the left costal margin) and slight liver enlargement. Blood: Hgb. 8.38 Gm., WBC 320,000, immature myeloid leukocytes‡ 34 per cent. Urethane, 3 Gm. daily in solution orally, was started on December 14, 1946, and was increased to 4 Gm. daily five days later. By the forty-third day of treatment

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* Prepared according to our specifications regarding sterility, isotonicity and pH correction by Dr. F. B. Peck, Eli Lilly Laboratories.

† The term "x-ray resistance" is here employed without implications of any kind except to designate the fact that our own Department of Roentgenology, employing the technics and dosages judged by this Department to be the best under the circumstances, had failed to obtain a remission from one or more recent courses of therapy which formerly in the same patient had produced a remission.

‡ The term "immature myeloid leukocytes" refers to the combined percentage of myelocytes, promyelocytes, and myeloblasts.

the spleen measured 11 cm and the blood count was Hgb 9 9 Gm WBC 11 000 immature myeloid leukocytes *none* The drug was continued until March 22 1947 (ninety-eighth day) when the spleen *measured 3 cm and the blood showed Hgb 13 0 Gm WBC 10 000 immature myeloid leukocytes 3* per cent The patient was symptom free

This patient conceived while on urethane therapy When last seen (Aug 26 1947) she was asymptomatic vigorous and in the seventh month of pregnancy The spleen was palpable 6 cm below the costal rim Blood Hgb 11 4 Gm WBC 35 000 immature myeloid leukocytes 7 per cent

Case 9 White female age 38 Urethane was first administered during the late stage of chronic myelogenous leukemia of over six years duration The patient had failed to respond to her most recent (seventh) *course of irradiation* She showed cachexia lymphadenopathy and an enormous spleen which al

TABLE 2 —*Chron c Myel g rous Le k mia*

Cas	O g l Stat s	WBC Start U	U (g m) U (days)	WBC End U	R ult
#4 W 30 F	Untreated	310 000	256 98	10 000	Good Pregnant
#9 W 38 F	Duration 6 years X ray resistant	149 000	208 57	76 000	Died during treatment
#10 W 46 M	Duration 3 years	103 000	13 13	9 000	Relapsed in 2 weeks Died
#17 W 65 M	Duration 1 year	43 000	17 17	20 000	Stopped U because of nausea Died 2 mo later
#20 W 50 F	Duration 4 years X ray and P32 resistant	395 000	28 7 (I V)	220 000	Died during treatment
#22 W 63 M	Duration 2 years Fowler s solution	55 000	29 20 (I V)	14 000	Poor Given x ray therapy Died
#24 W 51 F	Duration 4 years X ray therapy in remission	30 000	116 44	5 100	Good

most filled the abdomen Blood Hgb 7 97 Gm WBC 149 000 immature myeloid leukocytes 34 per *cent* Urethane 4 Gm daily in solution *orally* was started on October 25 *1946* and continued until the patient expired on December 23 1946 Blood Hgb 8 02 Gm WBC 76 000 immature myeloid leukocytes 31 per cent

Case 10 This white male age 46 was a terminal much t eated case of chronic myelogenous leukemia of o er three years duration He was readmitted to the hospital in an acute relapse phase of his disease Urethane 1 Gm daily in solution or lly was given for thirteen days with a fall in the leukocyte count from 103 000 to 9 000 *No clinical improvement occurred and the patient died within two weeks after* stopping the drug

Case 17 A white male age 65 was a terminal case of subacute myelogenous leukemia of one year du ation resistant to irrad ation He was unable to tolerate an adequate dose of urethane either orally or by suppository A leuk m c tumor of his forehead shrank in s ze and finally disappeared after application of 50 per cent ureth ne aquaphor ointment He died soon thereafter

Case 20 This white female age 50 was a leukemic of four years duration After numerous courses of irradiation she was finally considered x ray resistant She was cachectic anasarcatous and exhibited massive enlargement of lymph nodes spleen and liver Blood Hgb 4 95 Gm WBC 395 000 immature myeloid leukocytes 52 per cent Urethane 2 Gm then 4 Gm daily intravenously was started May 25 1947 and continued until she died suddenly on June 1 1947 soon after thoracentesis On May 31 1947 she had been clinically unimproved although her white blood count had dropped to 22 000 on this date

Case 22 This white male age 63 had suffered weakness and weight loss for three years He had just completed a short course of Fowler s solution when first examined on June 13 1947 He presented fever

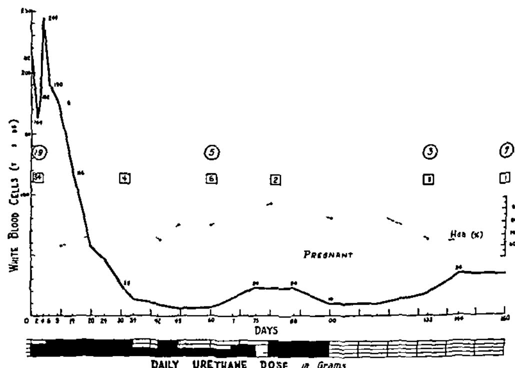


Fig 1 Case 4 Chronic Myelogenous Leukemia in a White Female Age 30 (□ = Per Cent Blasts Promyelocytes and Myelocytes ○ = Splenic Size in cm)

pallor petechiae edema and moderate splenic and lymph node enlargement Blood Hgb 7 59 Gm WBC 63 000 immature myeloid leukocytes 33 per cent Platelets 45 000 Intravenous urethane 2 Gm was given on June 19 1947 (WBC 55 000) and was increased to 3 Gm daily the following day On June 29 1947 the drug was stopped after a total of 29 Gm intravenously The patient was unimproved Blood Hgb 7 5 Gm WBC 14 000 immature myeloid leukocytes 13 per cent Tran fusions were ineffective Radioactive phosphorous was given but the patient died within two week

Case 24 This white female age 51 was known to have leukemia of four years duration She had had several excellent x ray induced remissions When seen in the clinic May 8 1947 she complained of recent epistaxis and weakness Her general condition seemed good The lymph nodes and spleen were not enlarged Blood Hgb 12 4 Gm WBC 50 000 immature myeloid leukocytes 17 per cent Platelets 142 000 Over the next forty four days a total of 116 Gm urethane in solution orally was administered On June 27 1947 she felt entirely well Blood Hgb 13 2 Gm WBC 5 100 immature myeloid leukocytes 2 per cent Platelets 256 000 When last seen Aug 11 194 she was clinically well Blood Hgb 13 7 Gm WBC 26 000 immature myeloid leukocytes 10 per cent

Chronic Lymphatic Leukemia

Our results in 7 cases of chronic lymphatic leukemia are summarized in table 3 It is of interest that the first case we treated with urethane (Case 1) has now enjoyed an entirely satisfactory remission of slightly over one year s duration

CASE REPORTS

Case 1 This white female age 57 first noted weakness and left sided heaviness in May 1946 When first examined by us on June 20 1946 massive splenomegaly and slight lymphadenopathy were found Blood Hgb 12 2 Gm WBC 700 000 lymphocytes 96 per cent Platelets 118 000 Urethane 2 Gm daily in solution orally was started on June 24 1946 and increased to 4 Gm daily eleven days

TABLE 3 —*Chronic Lymphatic Leukemia*

C	O sl St s	WBC Start	L (r m) L (d)	WBC End	R ult
#1 W 57 F	Untreated	700 000	273 72	4 500	Good Remission 362 days plus
#3 W 54 F	D ration 1 year 1 course x ray therapy	102 000	191 76	6 400	Good Remission 184 days plus
#6 W 59 M	Untreated	58 000	683 299	21 000	Poor
#7 W 67 M	Duration 1½ years X ray 7 months before treat ment	233 000	225 6	8 700	Hem l Good Clin Poor Died at 75 days
#8 W 61 M	Untreated	238 000	297 63	293 000	Poor Gi en x ray ther apy Fair response
#13 W 52 M	Untreated (Leukemia Cu tis)	44 000	521 136	26 000	Poor
#16 W 45 M	Untr ated	265 000	218 63	22 000	Poor Hypoplastic mar row

later Marked shrink ge in size of the spleen was apparent after forty three days of treatment On September 3 1946 (seventy second d y of treatment) after a total of 283 Gm of urethane treatment was stopped The patient was entirely s mptom free the spleen as barely palpable Blood Hgb 11.41 Gm WBC 4 500 lymphocytes 53 per cent When last seen on August 29 1947 the patient was in good condition The spleen ag in showed slight enlargement Blood Hgb 12.4 Gm WBC 32,000 lymphocytes 92 per cent

Case 3 This hite fem le age 54 w found to have asymptomatic chronic lymphatic leukemia in September 1945 and a brief course of irradiation therapy was given She was first seen in this clinic December 7 946 She was still asymptomatic there was no lymph denopathy and the spleen was moderately enlarged Blood Hgb 12 95 Gm WBC 10 000 lymphocytes 87 per cent Urethane 3 Gm daily in sol tion orally was started The drug was stopped on February 16 1947 (seventy s xth day of tre tment) after a total dose of 19 Gm her spleen w s impalpable she was still symptom free and her blood count w s Hgb 12 5 Gm WBC 6 400 lymphocytes 66 per cent

The drug was resumed on April 27 1947 because of a rise in the leukocyte count to 19 800 On May 19 1947 after a total dose of 69 Gm urethane was again discontinued when her blood count was Hgb 12 0 Gm WBC 6 200 lymphocytes 40 per cent When last seen on August 15 1947 the patient was still asymptomatic her spleen was barely palpable and her blood count was Hgb 33 42 Gm WBC 11 000 lymphocytes 79 pe cent

Case 6 This white male age 59 was found in a routine blood count to have 43 000 leukocytes with 62 per cent lymphocytes He was a ymptomatic with shotty lymphadenopathy and a barely palpable liver and spleen Although he required no specific treatment at this time urethane was started on July

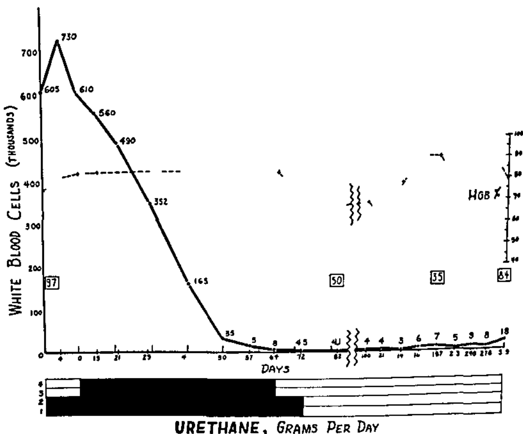


FIG 2 CASE 2 CHRONIC LYMPHATIC LEUKEMIA IN A WHITE FEMALE AGE 57
(□ = PER CENT LYMPHOCYTES)

27 1946 in a dosage of 2 Gm daily in solution orally Blood Hgb 14 5 Gm WBC 58 000 lymphocytes 89 per cent No effect of the drug could be detected after 299 days of treatment (total dose 683 Gm) and it was stopped on May 15 194 when the blood count was Hgb 13 0 Gm WBC 21 000 lymphocytes 93 per cent

This case suggests that low grade chronic lymphatic leukemia is resistant to urethane in a dosage of 2 Gm daily

Case 7 This white male age 67 was hospitalized in March 1944 for prostatic resection At this time the blood picture of chronic lymphatic leukemia was found He received a course of irradiation therapy in October 1945 When first seen in the clinic on November 12 1946 he was acutely ill with findings of massive lymphadenopathy splenomegaly and marked pulmonary infiltration Blood Hgb 7 5 Gm

WBC 233 000 lymphocytes 99 per cent Platelets 320 000 Urethane 4 Gm daily in solution orally was started on November 16 1946 On January 18 1947 (sixty-second day of treatment) after a total dose of 225 Gm the drug was stopped when the blood count was Hgb 6 9 Gm WBC 5 600 lymphocytes 93 per cent The patient had become progressively worse He died in cardiac failure on January 31 194 despite apparent improvement in the abnormal lymphocytosis

Case 8 This white male age 61 had sudden severe pain in the left side of the abdomen in November 1946 Examination disclosed generalized lymphadenopathy marked splenomegaly and marked lymphocytosis Blood Hgb 11.4 Gm WBC 238 000 lymphocytes 98 per cent Urethane was started on November 9 1946 2 Gm daily in solution orally Dosage was increased to 4 Gm daily on November 27

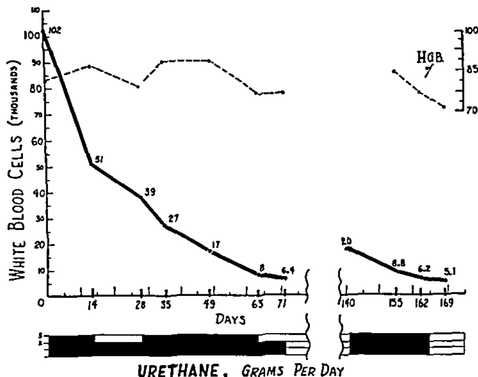


Fig 3 Case 3 Chronic Lymphatic Leukemia in a White Female Age 54

1946 and to 6 Gm daily on December 5 1946 Weakness marked sweating and insomnia developed On January 6 1947 lymph nodes were estimated 80 per cent reduced in size but the size of the spleen was unchanged Blood Hgb 11 1 Gm WBC 400 000 The dosage of urethane was decreased to 4 Gm daily until January 20 1947 (sixty-third day of treatment) when it was discontinued (total dose 297 Gm) Blood Hgb 12 2 Gm WBC 293 000 lymphocytes 97 per cent X-ray therapy was instituted with prompt fall in the leukocyte count but little change in size of lymph nodes or spleen When last examined on August 25 1947 moderate splenomegaly and lymph node enlargement were found His blood count showed Hgb 13 6 Gm WBC 38 000 lymphocytes 93 per cent

This case is judged a urethane failure Although regression in lymph node size occurred there was no improvement in the splenomegaly or in the blood picture

Case 13 This white male age 52 presented generalized exfoliative dermatitis lymphadenopathy and lymphocytosis diagnosed as leukemia cutis of six months duration Urethane 4 Gm daily in solution

orally was started on January 7 1947 Blood Hgb 13 0 Gm WBC 44 000 lymphocytes 83 per cent No change in the patient s clinical condition was apparent when the drug was stopped 136 days late (total dose 521 Gm) Blood Hgb 11 5 Gm WBC 21 000 lymphocytes 33 per cent

TABLE 4 —*Acute Leukemia*

Case	*Original Status*	*WBC Start U*	*U (grams) U (days)*	*WBC End U*	*Result*
#2 W 50 F	Untreated Monocytic	35 000	60 14	1 100	Good initial but relapse 3 mo
#5 W 33 F	Untreated Myelogenous	79 000	26 10	8 000	Poor Died
#11 W 14 F	Untreated Myelogenous	240 000	64 20	250 000	Poor Died
#12 W 12 F	Untreated Aleukemic Lymph	1 800	14 7	900	Poor Died Aplastic marrow
#14 W 42 M	Untreated Myelogenous	77 000	48 8	49 000	Poor Died
#15 W 15 M	Untreated Myelogenous	221 000	137 49	149 000	Poor Died 1 mo after treatment
#18 C 3 F	Untreated Myelogenous	56 000	26 13	300	Fair for 1 mo then relapse Marked decrease in size of kidney masses
#19 C 38 M	Untreated Myelogenous	110 000	6 17 (I V)	9 500	Left hospital No follow up
#21 W 36 M	Untreated Lymphatic	200 000	28 14 (P O)	39 000	Poor Died
		39 000	106 24 (I V)	132 000	
#23 W 5 F	Untreated Myeloblastic	132 000	3 2 (I V)	2 00	Poor Died

Case 16 This white male age 45 noted enlargement in circumference of neck in December 1946 When first seen on January 18 194 he presented generalized lymphadenopathy and moderate splenomegaly Blood Hgb 13 99 Gm WBC 265 000 lymphocyte 93 per cent Platelets 112 000 Urethane 3 Gm daily orally first in solution then in capsules was started on January 27 194 The dose was soon increased to 4 Gm daily By March 20 194 (fifty first day of treatment) dangerous symptoms of drowsiness weakness anorexia fever petechiae mucosal bleeding and hematuria appeared Blood Hgb 52 Gm WBC 51 000 lymphocytes 93 per cent Platelets 50 000 On April 1 1947 (sixty third day of treatment) after a total of 218 Gm the drug was stopped No significant changes in lymph node or splenic size were

demonstrable Multiple transfusions followed by cautious x ray irradiation produced slow improvement with cessation of hemorrhagic phenomena and marked reduction in the size of lymph nodes The blood picture improved and when last seen on August 25 1947 his blood count showed Hgb 13 20 Gm WBC 81 000 lymphocytes 79 per cent Platelets 64 000

This case is obviously another urethane failure It is possible that the drug produced critical hypoplasia of erythrocytic and megakaryocytic elements in the bone marrow

Acute Leukemias

Table 4 summarizes the results in 10 cases of acute leukemia of various types In most cases a prompt fall in the total leukocyte count followed urethane treatment Evanescent clinical improvement occurred in 4 patients (Cases 2 15 18 19) Final information regarding 2 patients in this series is not available All other patients died during or soon after urethane therapy

CASE REPORTS

Case 2 This white female age 40 had acute monocytic leukemia (Naegeli type) with fever gingival necrosis and extensive intracutaneous and mucosal bleeding of 2 weeks duration Urethane 4 Gm daily in solution orally was started on August 21 1946 Blood Hgb 7 2 Gm WBC 35 000 immatures (promonocytes and monoblasts) 64 per cent A total of 60 Gm was given during the next ten days with rapid fall in leukocytes to 1 100 then to 650/cu mm Progressive anemia paralleled the fall in leukocytes On September 44 1946 the blood count showed Hgb 1 38 Gm WBC 1 300 immatures 26 per cent Despite these desperately low blood levels the patient felt improved the gums healed and bleeding ceased There was gradual improvement and when the patient was sent home on October 45 1946 the blood count was Hgb 4 95 Gm WBC 2 400 immatures 36 per cent

It is noteworthy that sternal aspiration soon after termination of therapy showed striking reduction in numbers of blast cells as compared with the original aspiration

Continued improvement was maintained On December 4 1946 the blood count showed Hgb 10 4 Gm WBC 4 700 immatures none (commercial clinical laboratory) In mid January 1947 the patient fell injured her mouth and apparently was precipitated into an acute relapse Urethane 4 Gm in solution orally was resumed on January 23 1947 when the blood count was Hgb 9 76 Gm WBC 70 000 immatures 69 per cent The patient developed diffuse bronchopneumonia and died February 6 1947 Blood Hgb 2 9 Gm WBC 2 300

This was our first experience with the swift effect of urethane on the leukocyte count observed subsequently in most of our cases of acute leukemia The partial remission in this patient encouraged further trials in this form of leukemia

Case 5 White female age 53 Acute myelogenous leukemia Urethane 2 to 4 Gm daily in solution orally was started on June 30 1946 when the blood showed Hgb 12 4 Gm WBC 79 000 immatures (promyelocytes and myeloblasts) 82 per cent Four days later the patient exhibited a phlebothrombosis of the right leg On July 9 1946 urethane was discontinued (total dose 26 Gm) Blood Hgb 9 2 Gm WBC 8 000 On July 23 1946 the leukocyte count rose to 31 000 and urethane 4 Gm daily orally was resumed The patient died suddenly of pulmonary embolism July 31 1946 after a second course of 20 Gm of urethane when the blood count was Hgb 7 9 Gm WBC 16 000

Case 11 White female age 14 This patient had acute myelogenous leukemia of two weeks duration with generalized lymphadenopathy hepato-splenomegaly gingivitis mucosal and intracutaneous bleeding On December 44 1946 the blood count showed Hgb 12 2 Gm WBC 360 000 blast cells 89 per cent A total dose of 68 Gm of urethane was given between December 30 1946 and January 7 1947 when the patient died after a progressively downhill course Blood Hgb 6 2 Gm WBC 250 000

Case 2 White female age 12 This patient had acute aleukemic lymphatic leukemia with hemolytic staphylococcus aureus septicemia Urethane 4 Gm daily in solution orally was started on April 7 1947

Blood Hgb 6 27 Gm WBC 1 800 blast cells 82 per cent Urethane was discontinued April 14 1947 (total dose 14 Gm) when the blood count showed Hgb 6 6 Gm WBC 900 Death occurred April 22 1947 despite multiple transfusions and penicillin therapy

Sections of marrow obtained at autopsy showed almost complete aplasia in contrast to the initial sternal marrow appearance of acute lymphoblastic leukemia

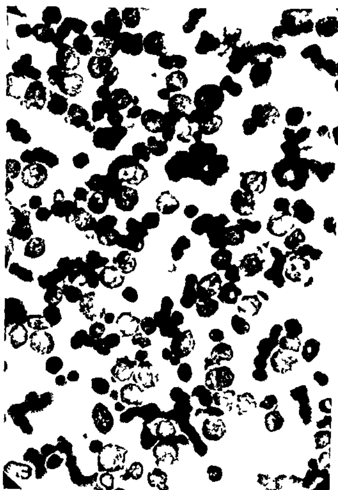


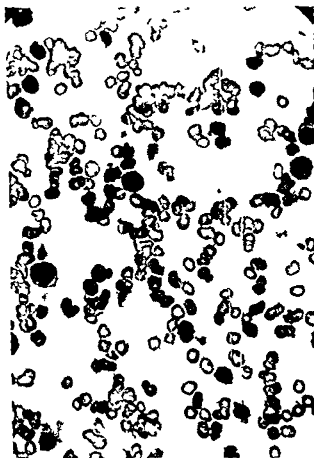
FIG 4 BONE MARROW OF CASE 2 BEFORE TREATMENT

Case 14 White male age 42 This patient had acute myelogenous leukemia and was moribund on admission He was given 6 Gm urethane daily in solution orally for eight days without detectable effect clinically or hematologically The patient died August 2 1947 while still under therapy

Case 15 White male age 15 Acute myelogenous leukemia He had received 115 Gm urethane orally in 32 days while in another hospital with a fall in leukocytes from 221 000 to 19 300 but without clinical improvement

Urethane 3 to 4 Gm daily orally in solution was instituted March 1 1947 Blood Hgb 13 8 Gm WBC 74 000 immatures (blasts) 96 per cent Because of severe nausea vomiting and diarrhea the drug was stopped March 8 1947 (total dose 141 Gm) WBC 125 000 Irradiation therapy was started March 10 1947 and given daily until discharged at his request on March 19 1947 when his blood count showed Hgb 7 4 Gm WBC 173 000 This patient died two weeks later

Case 18 Colored female age 3 Acute myelogenous leukemia This patient had been ill for one month The outstanding physical finding was the presence of huge bilateral renal masses Urethane 2 Gm daily in solution orally was started March 12 1947 when the blood count was Hgb 4 3 Gm WBC 41 000 immatures (blast cells) 40 per cent By March 19 1947 the renal masses had decreased markedly in size (by palpation and urography) The drug was stopped on March 24 1947 when the blood count showed Hgb 4 6 Gm WBC 1 100 immatures none The patient s med t mporarily impro ed but relapsed and died one month later



F G 5 BONE M RROW OF CASE 2 AFTER TREAT ENT

Cas 9 Colored male age 38 Acute myelogenous leukem a Urethane 3 Gm intravenously was st rted on May 9 1947 and increased to 4 Gm daily the following day Blood Hgb 2 11 Gm WBC 110 000 immatu es (promyelocytes and m eloblasts) 9 per cent On May 25 1947 after a total of 67 Gm intra enously the p tient showed impro ement in strength and a o per c nt d cr a e in the size of the lymph nodes The blood count at this time ho ved Hgb 5 9 Gm WBC 9 500 immatures 73 per cent After recei ng 2 000 cc of whole blood the patient as discharged June 10 1947 when the blood count showed Hgb 7 8 Gm WBC 5 500 immatures 69 per cent It has b 2 impo sible to obtain further information rega ding th s patient

This ase observed at Philadelphia Gen ral Ho pital court sy of Dr Paul Gyorgy

Case 21 White male age 30 Acute lymphatic leukemia This patient had been given 28 Gm of urethane in fourteen days elsewhere with fall in leukocyte count from 200 000 to 39 000 He was admitted to the Hospital of The University of Pennsylvania on May 26 1947 in poor condition and given preliminary dose of 3 Gm urethane intravenously at which time the blood count was Hgb 8 8 Gm WBC 21 000 immatures (prolymphocytes and lymphoblasts) 55 per cent The dose was changed to 4 Gm daily on May 27 1947 and again to 6 Gm daily on June 13 1947 Blood Hgb 6 9 Gm WBC 109 000 immatures 99 per cent A total of 106 Gm urethane intravenously had been given over a twenty four day

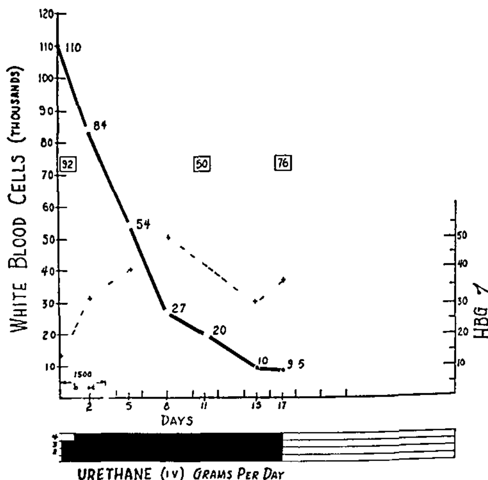


FIG 6 CASE 19 ACUTE MYELOGENOUS LEUKEMIA IN A NEGRO MALE (□ = PER CENT BLASTS AND PROMYELOCYTES)

period when this patient died June 20 1947 The blood count then was Hgb 8 6 Gm WBC 132 000 The patient had also received 5 000 cc of whole blood during his hospital stay

Case 23 White female age 5 Acute myelogenous leukemia One Gm urethane intravenously was given June 8 1947 when the blood count was Hgb 7 02 Gm WBC 132 000 blasts 90 per cent On June 9 1947 2 Gm urethane was given intravenously This was followed by a sudden rise in temperature to 105 2 F rectally Urethane therapy was discontinued On June 12 194 marked diminution in splenic size was noted and the blood count was Hgb 6 13 Gm WBC 2 700 The patient died June 17 1947 with a Hgb of 4 75 Gm and a WBC of 750 cu mm

Summary of Results

It is apparent from the data presented above that satisfactory remissions as a result of urethane therapy occurred in 4 cases (1 3 4, 24) of our combined number of 24 cases It is worthy of emphasis that of these 24 cases at least 14 (Cases 9 10 17 20 and all acute leukemias) and possibly 2 others (Cases 7 and 21) would in our opinion have proved hopelessly refractory to any known method of treatment of leukemia It may be significant also that although urethane was withdrawn in Cases 8 and 13 because of poor response these patients subsequently responded poorly to irradiation and radioactive phosphorous respectively

If the experimentally treated 4 terminal cases of chronic myelogenous leukemia are omitted from consideration it is apparent that urethane produced a satisfactory result in 2 of the 3 remaining cases Adequate response was obtained in 2 of 7 cases of chronic lymphatic leukemia It becomes evident that of 10 fair cases of chronic leukemia urethane was successfully used in 4

Drug Effects

Toxicity Nausea has been the predominant symptom of intolerance Approximately 50 per cent of our patients exhibited mild or severe nausea commencing at any time during the course of oral administration of the drug In a few cases nausea was sufficiently severe to warrant interruption or cessation of treatment Lesser toxic manifestations have been vomiting anorexia excessive sweating diarrhea and drowsiness

The most dangerous toxic manifestation has been the appearance of evidence suggesting depression of all marrow elements (Cases 12 16) Whether or not urethane produced or contributed to this effect it is important to be wary of abrupt falls in erythrocyte or leukocyte levels during the period of urethane administration It is significant that 2 patients in Paterson s series exhibited similar hypoplastic changes Particular caution is advisable when leukopenic leukemia is under treatment on the basis of Case 12 Rapidly developing anemia in our experience calls for immediate cessation of urethane therapy

Urethane administered intravenously has produced only minimal side effects such as transitory drowsiness and transitory elevation of temperature No local irritative phenomena have been observed Particularly gratifying has been the absence of nausea suggesting that the occurrence of this symptom after oral administration is due to gastric irritation It is possible that enteric coated capsules or tablets may circumvent such nausea and trials with such preparations are in progress

Rapidity of effect The time required for a definite reduction in the leukocyte count (to 15-20 000) in favorable chronic cases averaged forty-eight days when the drug was given by mouth In the acute leukemias the effect was much more rapid averaging ten days It is believed that intravenous administration of urethane will produce effects more quickly than the above mentioned periods although our data are as yet insufficient to establish this point

Mode of action The manner and site of action of urethane are entirely unknown

Case 21 White male age 30 Acute lymphatic leukemia This patient had been given 28 Gm of urethane in fourteen days elsewhere with fall in leukocyte count from 200 000 to 39 000 He was admitted to the Hospital of The University of Pennsylvania on May 26 1947 in poor condition and given preliminary dose of 3 Gm urethane intravenously at which time the blood count was Hgb 8 8 Gm WBC 21 000 immatures (prolymphocytes and lymphoblasts) 55 per cent The dose was changed to 4 Gm daily on May 27 1947 and again to 6 Gm daily on June 13 1947 Blood Hgb 6 9 Gm WBC 109 000 immatures 99 per cent A total of 106 Gm urethane intravenously had been given over a twenty four day

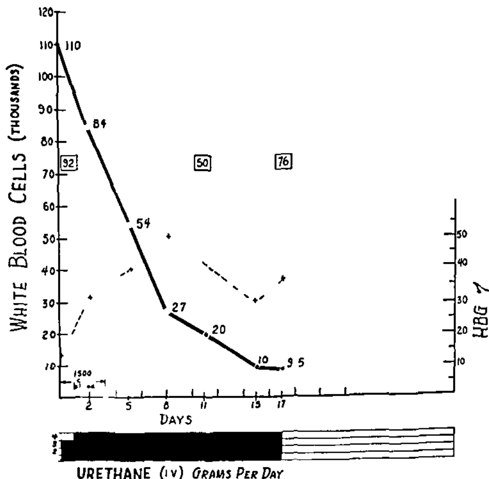


Fig 6 Case 19 Acute Myelogenous Leukemia in a Negro Male (☐ = Per Cent Blasts and Promyelocytes)

period when this patient died June 20 194 The blood count then was Hgb 8 6 Gm WBC 132 000 The patient had also recei ed 5 000 cc of whole blood during his hospital stay

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workers that urethane and irradiation therapy are not mutually exclusive in effect Our evidence does indicate strongly that urethane is without clinical effect in terminal long standing repeatedly irradiated cases of chronic leukemia

It is our impression that urethane will prove to be an occasionally useful adjunct in the management of chronic leukemia The low cost and convenience of administration in comparison with irradiation therapy are strong points in favor of this drug

It is the opinion of the authors that in the treatment of leukemia in general urethane is less consistent in effect than standard methods of irradiation therapy and more consistent and more efficient than such modalities as Fowler s solution benzol and colchicine

Our results while not as striking generally confirm the findings reported by Paterson and her co-workers Urethane deserves further trial in the treatment of leukemias of all types

SUMMARY

1 The results of urethane therapy in 24 cases of leukemia are described

2 The average daily dose is 4 Gm orally or intravenously

3 The drug is irregularly effective Chronic myelogenous leukemia appears more responsive than the lymphatic variety

4 Acute leukemias are not significantly altered in course by urethane

5 Urethane produces a fall in the total leukocyte count in a majority of all types of leukemia Clinical improvement does not necessarily follow

6 Nausea is the most frequent side effect of urethane therapy Possible marrow aplasia is the most dangerous toxic effect

7 Urethane is of definite but of limited value in the treatment of chronic leukemia In some instances it compares favorably with x ray therapy but in general it is less dependable particularly in its frequent failure to induce optimum return of normal red cell and platelet values and optimum regression of organ infiltration

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[1] HADDOW A AND SEXTON W A Influence of carbamic esters (urethane) on experimental animal tumors Nature 15 500 1946

[2] PATERSON E HADDOW A THOMAS I A AND WATKINSON J M Leukemia treated with urethane Lancet 250 6— 1946

[3] ARCHER H E CHAPMAN L RHODEN E AND WARREN F L The Estimation of Urethane (Ethyl Carbamate) in Blood Biochem J 41 No 2 p xxxi 194

Haddow and Sexton[1] offer speculation regarding the possible action of urethane in remedying some deficiency in the process of leukocytic maturation in leukemia

Basal metabolism and blood chemistry In a small number of patients with chronic forms of leukemia we observed that the basal metabolic rate fell in proportion to the decline in the total white cell count during urethane therapy

Marked reduction in elevated levels of blood uric acid was also noted in a few of our cases during therapy Case 19 had a blood uric acid of 11 3 mg per 100 ml on admission which fell to 3 8 mg per 100 ml at the end of urethane therapy when the white blood count was 8 000 Case 4 had a reduction of blood uric acid from 8 9 mg per 100 ml to 4 2 mg per 100 ml while under urethane therapy

Blood urea nitrogen determinations were made before during and after urethane administration in 10 of our cases Elevation of the level of blood urea nitrogen occurred in no case during full urethane dosage

Blood levels of urethane have not been obtained A method of estimation has recently been published by Archer et al [3]

Discussion

Urethane adequately administered in cases of chronic luekemia frequently produces a marked reduction in the total leukocyte count (83 3 per cent of our series) In fully responsive favorable cases definite clinical improvement gradually follows Splenomegaly is reduced enlarged lymph nodes become smaller and a feeling of betterment is expressed The hemogram assumes a more normal appearance in its entirety Unfortunately in a larger number of cases the fall in the leukocyte count is a spurious improvement In such cases visceral pathology and anemia may fail to improve or may grow worse and the patient may exhibit progressive deterioration Continued administration of the drug in such instances may lead to rapid clinical deterioration It is generally advisable to suspend the drug if clinical improvement fails to appear within one week after a satisfactory rate of fall of the leukocyte count has appeared It is furthermore probably advisable to discontinue urethane if no significant fall in the white cell count has occurred within sixty days from the start of treatment

It is impossible from our small series of cases to offer definite criteria for selection of patients for urethane treatment It seems likely (in agreement with Paterson et al [2]) that chronic myelogenous leukemia responds better to urethane than does the lymphatic variety The drug has proved of little value in the acute leukemias although further trials are justifiable in view of the swiftly fatal outcome of this form of leukemia

No information is as yet available as to the possibility of inducing repeated remissions in chronic leukemia with urethane and if so whether these can then be followed by successful repetitions of x ray therapy If this should prove the case then prolongation of the leukemic life span as well as amelioration of symptoms may be possible In this regard it may be mentioned that in Cases 3 and 24 urethane successfully induced remissions after relapse from previously successful x ray therapy Conversely in 2 other cases (8 and 16) x ray therapy successfully followed urethane failures This is in accord with the observation of the British

intervals thereafter for five days The criteria of cell identification and classification were those given and illustrated by Osgood and Ashworth [7]

OBSERVATIONS

The morphologic changes in structure of the cells of the granulocyte series noted in the cultures containing urethane are illustrated in figure 1 and outlined in table 1

The first change to appear was a 5 to 10-fold increase in the number of progranulocytes in process of mitotic division as compared to the control This was noted as early as three hours and in concentrations as low as 1:40,000 The increase in mitoses was most marked at twenty four hours and persisted for seventy two hours All phases of mitotic division were seen but most were normal appearing metaphases similar to those illustrated in figure 2 by Osgood [8]

The most commonly observed change was the condensation of the basichromatin in the nucleus of the progranulocytes and granulocytes into dense blocks separated by clear spaces and still surrounded by a nuclear membrane as shown in figure 1 a This appeared as early as three hours steadily increased as long as the cultures were observed and was most marked in the higher concentrations although it was noted with all concentrations

The alteration in morphology shown in figure 1 b was frequently observed in the progranulocyte and granulocyte stage There seemed to be a loss of the nuclear membrane and imperfectly square or rectangular projections from a mass of clumped chromatin A somewhat similar picture is seen in the anaphase of normal mitotic division when the cell is so oriented that the plane of cell division is parallel to the plane of the slide but the number of these cells was greater than would be expected from the number of cells seen in the metaphase of division and it seems probable that at least some of the cells showing this appearance represent an intermediate stage of karyorrhexis between figure 1 a and figure 1-c

The most striking but least frequently observed change is shown in figure 1-c It consisted of the appearance within the cell of numerous fragments of densely-staining structureless chromatin in round ovoid or rectangular blocks with no nuclear membrane This appearance was somewhat suggestive of the colchicine arrest of mitosis in the metaphase [9] illustrated in figure 1 in Osgood [10] but careful studies showed the points of difference outlined in table 2 It seems more probable therefore that the urethane effect illustrated in figure 1-c represents karyorrhexis or fragmentation of a nucleus similar to that in figure 1 a

Double nuclei with no suggestion of fission of the cytoplasm were frequent in the progranulocyte and all subsequent stages of the granulocyte series in the urethane-containing cultures yet the cells did not appear to be appreciably larger than the corresponding cell type with a single nucleus They seemed to be most abundant in the granulocyte stage but appeared first in the progranulocyte stage as illustrated in figure 1-d

Another change in morphology which is not illustrated was a marked decrease in size of some of the neutrophil lobocytes and rhabdocytes both as compared to the control culture and to the average normal size of these cells This change was even more noticeable in the blood of some of the patients treated with urethane over long periods of time

THE EFFECT OF URETHANE ON THE NUCLEAR MORPHOLOGY OF CELLS OF THE GRANULOCYTE SERIES AS OBSERVED IN MARROW CULTURES AND LEUKEMIC BLOOD

By EDWIN E OSGOOD M D AND I T CHU M D

INTEREST in urethane (NH CO OC_2H_5) and related carbamic acid esters in the treatment of metastatic malignant tumors and leukemias has been greatly stimulated by the report of Haddow and Sexton[1] on the effects of urethane on tumors in experimental animals and of Paterson Haddow, Thomas and Watkinson[2] on urethane therapy of human leukemias and malignant tumors They demonstrated striking decreases in the leukocyte count and spleen size in certain patients with granulocytic or lymphocytic leukemias and in the size of metastatic nodules in a small proportion of patients with other malignant tumors The observations of Paterson Haddow Thomas and Watkinson have been confirmed by Goodman and Lewis[3] and by the authors at the University of Oregon Medical School

Although urethane has long been in use as a narcotic and there is much literature on the action of the urethanes and related karyoklastic and karyokinetic poisons on the cells of lower forms of life [4] we were unable to find any reference to morphologic changes produced by urethane in the nuclei of human cells

The present study using the technic of human marrow culture [5] was undertaken as part of a long term investigation of the factors influencing the fundamental phases of cell growth namely increase in size mitotic and amitotic division differentiation and life span This paper will be limited to the morphologic changes in cells of the granulocyte series produced by the action of urethane Quantitative data on the comparative effects of urethane and methyl bis (B chloroethyl) amine hydrochloride obtained in the course of this study will appear later

METHOD

Marrow cultures were set up as previously described [6] using sterile ascitic fluid as a source of protein instead of human cord serum A marrow culture was prepared from each of 8 patients with miscellaneous diseases displaying essentially normal marrow pictures In addition the bloods of 4 patients with chronic granulocytic leukemia 2 with acute lymphocytic leukemia 1 with acute monocytic leukemia and 1 with multiple myeloma with a plasmacytic leukemia blood picture were cultured in the same way as marrow Each culture was first thoroughly shaken in one vial and then equal parts were transferred to a series of vaccine vials one of which was left as a control to which the solvent only was added and to the others equal volumes of varying concentrations of urethane or of methyl bis (B-chloroethyl) amine hydrochloride in isotonic saline were added Since no references were found giving the actual blood levels of urethane obtained in clinical therapy a wide range of final concentrations was used including 1 200 1 1,000 1 2 500 1 5 000 1 10 000 1 20 000 and 1 40 000 although not all concentrations were used in each experiment The morphologic data were based on the study of Wright s stained smears made at the same time from the control urethane and nitrogen mustard cultures The samples for the smears were removed three hours after the drugs were added and at approximately twenty four hour

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From the Division of Experimental Medicine University of Oregon Medical School Aided by a grant from the Medical Research Foundation of Oregon

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OBSERVATIONS

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Double nuclei with no suggestion of fission of the cytoplasm were frequent in the progranulocyte and all subsequent stages of the granulocyte series in the urethane-containing cultures yet the cells did not appear to be appreciably larger than the corresponding cell type with a single nucleus They seemed to be most abundant in the granulocyte stage but appeared first in the progranulocyte stage as illustrated in figure 1-d

Another change in morphology which is not illustrated was a marked decrease in size of some of the neutrophil lobocytes and rhabdocytes both as compared to the control culture and to the average normal size of these cells This change was even more noticeable in the blood of some of the patients treated with urethane over long periods of time

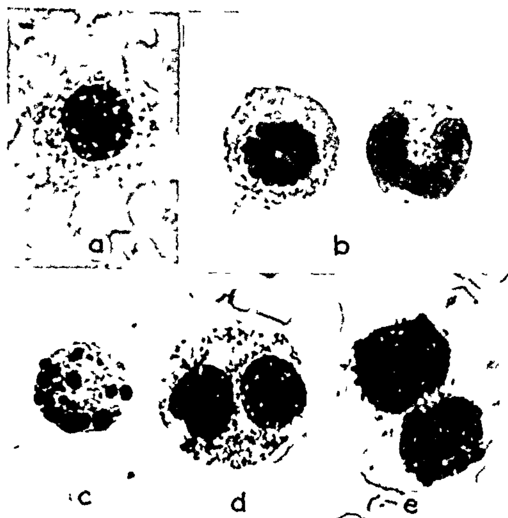


Fig 1 Photomicrographs of cells of the granulocyte series from urethane-containing culture (a d) and leukemic blood (e) Wright stain × 1800

a Neutrophil granulocyte showing chromatin clumps within the nucleus

b On the left is a cell which is either a neutrophil progranulocyte S in the anaphase of mitotic division viewed end on or a neutrophil granulocyte showing an intermediate stage of karyorrhexis of the nucleus between figure 1 a and 1 c On the right is a neutrophil rhabdocyte of perfectly normal morphology

c Neutrophil granulocytes showing fragmentation or karyorrhexis of the nucleus into numerous deeply staining round or ovoid blocks of varying size

d Progranulocyte A with double nucleus

e This is a progranulocyte A from the blood of a patient with chronic granulocytic leukemia under treatment with urethane in process of amitotic division showing the mode of formation of the double nuclei Note the rectangular projections from the nucleus with rounded corners Amitotic division and double nuclei are almost never noted in the granulocyte series in either normal blood or marrow or blood or marrow from patients with leukemia who are not receiving urethane Note that there is no tendency toward fission of the cytoplasm

In addition many of the cells of the granulocyte series developed dense pyknotic structureless nuclei and showed signs of loss of the cell membrane and disintegration

None of these changes was noted in significant numbers in cells of the lymphocyte, monocyte or plasmacyte series cultured or in the granulocyte series in the control and nitrogen mustard cultures They have rarely if ever been observed by the authors in the course of an extensive study of the blood and marrow of patients with granulocytic leukemia or other diseases when urethane was not being given

In each of the cultures and with each of the concentrations of urethane employed many of the cells at each stage of development showed none of these changes and

TABLE 1 —*Approximate Percentage of Progranulocytes and Granulocytes Showing Morphologic Changes*

Changes observed	Control cultures 1 to 5 days	Cultures containing urethane	
		1 day	5 days
Normal	Over 99%	90%	Less than 20%
Mitoses	Less than 1%	7%	Less than 1%
Chromatin blocks (Figure 1 a)	Not seen	Less than 1%	60–70%
Separate chromatin blocks (Figure 1 c)	Not seen	Not seen	Less than 1%
Double nuclei (Figure 1 d)	Not seen or less than 0 1%	Less than 1%	15%

TABLE 2 —*Effects on Morphology of Cells Produced by Colchicine and Urethane*

	Colchicine	Urethane
Chromatin blocks	rectangular or square uniform in size number = or > normal no of chromosomes	round ovoid or with rounded corners unequal in size number < normal no of chromosomes
Mitosis	arrested in metaphase	initial stimulation goes on to completion
Amitosis	not affected	greatly increased
Differentiation	prevented in higher concentration (1 100 000)	markedly abnormal but occurs in 1 200
Cell size	giant forms common	dwarf forms common
Nuclei	enlarged	pyknotic

appeared indistinguishable in morphology from cells of the corresponding stage of development in the control cultures or in the original marrow or blood of the patient Such a normal appearing cell is illustrated by the neutrophil rhabdocyte in figure 1 b

All of the changes noted in the cultures were also noted in blood or marrow of patients receiving 1 gram of urethane three times daily The cell in figure 1-e is from the blood of a patient receiving urethane therapy It shows the formation of double nuclei apparently by amitotic division

Comment

The changes in morphology of the cells of the granulocyte series observed in blood and marrow cultures containing urethane suggest an effect of this drug on the state of aggregation or organization of the nucleoprotein within the cell nucleus a tendency to disrupt the nuclear membrane and to interfere markedly with the normal process of cell division and cell growth They resemble in many respects the changes described by Dustin [4] Burt [11] Piton [1] and Chodkowski[13] and others whose work is cited in the references herein given [1,2,4,11–13] as produced by a wide variety of unrelated karyoklastic poisons including many urethanes many narcotics and arsenical compounds which seem to act by altering the permeability and integrity of cell membranes and nuclear membranes and the state of colloid aggregation of the nuclear and cytoplasmic proteins

The morphologic effects produced by urethane were not seen in marrow cultures exposed to nitrogen mustard While some of the changes superficially resemble those produced by colchicine [5,9–10] there are important differences Colchicine in adequate concentration seems to arrest mitotic division completely whereas urethane apparently stimulates division at first and the majority of cells complete division and differentiation

In previous studies using the marrow culture technic of the action of ionizing radiation including 200 KV and 1 000 KV roentgen rays neutron rays and radioactive phosphorus it was shown[5,9,14–16] that in the dosage employed in treatment of leukemias each modality of ionizing radiation inhibited the onset of the next division mitotic or amitotic rather than actually killing cells In none of these experiments were morphologic changes similar to those noted in the urethane cultures observed nor was the initial increase in mitoses and in total cell count which occurred in the urethane cultures noted

We have confirmed the clinical observations of Paterson and her co workers that there is a great difference in clinical response of patients with apparently identical forms of leukemia to similar doses of urethane It so happened that bloods of 2 of these patients were selected for culture before urethane was administered Both patients were middle aged women with long standing chronic granulocytic leukemia with typical blood pictures Both had been treated until resistant with local x ray and not with the preferred total body irradiation at regular intervals Both had refused further x ray treatment Case 1 unit number 157378 who responded well to urethane had had a splenectomy several years previously because of the huge size of the spleen Case 2 unit number 157231 which failed to respond to urethane had a huge spleen reaching to the left iliac fossa 25 cm below the costal margin and extending across the midline In other respects they were as nearly alike as any two cases of chronic granulocytic leukemia could be Both were given 1 gram of urethane three times a day

In case 1 the leukocyte count had dropped from 33 800 to 7 700 after thirteen days of therapy totalling 36 grams and to 2 000 by the eighteenth day of therapy totalling 51 grams at which time the therapy was discontinued In the course of the next twelve days the leukocyte count dropped to 600 and although the urethane had been stopped it continued at this low level for many weeks during

which time stomatitis from the agranulocytosis was controlled with difficulty by penicillin therapy She then had a gradual reversion to a more normal leukocyte count

In case 2 the initial leukocyte count before therapy was oscillating between 50 000 and 90 000 and was still about 50 000 after 141 grams of urethane were given in the course of thirty nine days The differential cell count pattern was not significantly altered and the size of the spleen was unchanged Both patients had a good deal of nausea and some vomiting from the drug and it is possible that in case 2 some of the drug was lost in the vomitus or was not taken so during the last of the course the dose was increased to 4 grams daily still without effect This patient subsequently showed an excellent response to intravenous radioactive phosphorus therapy as far as leukocyte count and alteration in spleen size were concerned although requiring somewhat higher dosage than the average patient who had not had x ray therapy previously

In the cultures of these two bloods to which urethane was added however the morphologic changes observed were within the experimental limits of the method indistinguishable in character and percentage of cells involved suggesting that the clinical differences in response were due to differences in concentration of the active compound actually reaching the cell

Summary

In cultures by the marrow culture technic of human marrow and leukemic blood containing concentrations of urethane from 1 200 to 1 40 000 marked changes in the morphology of the cells of the granulocyte series were noted

These changes were not noted in the control nor in duplicate cultures containing the methyl bis (B chloroethyl) amine hydrochloride form of nitrogen mustard in concentrations from 1 500 000 to 1 40 000 000 nor were they noted in previous studies of cultures containing colchicine or exposed to 200 kilovolt or million volt x rays neutron rays or radioactive phosphorus nor in the bloods or marrows of patients with untreated chronic granulocytic leukemia of healthy individuals or of persons with miscellaneous diseases

The changes consisted of an early increase in number of normal mitoses in the progranulocytes a steadily rising percentage of granulocytes and progranulocytes showing condensation of the chromatin in the nucleus into dense fragments separated by clear spaces a progressive increase in the number of cells of the granulocyte series with double nuclei affecting all cells from the progranulocytes to the neutrophil lobocytes but appearing to be most numerous in the granulocyte stage and the appearance in the cultures by 4 to 5 days of cells containing separated fragments of structureless material staining like basichromatin which probably represents a karyorrhexis of the nucleus

Note Nothing in this article is to be construed as a recommendation of urethane for the clinical treatment of leukemias While many years must elapse before its place in therapy can be evaluated it does seem worthwhile to give urethane a trial for metastatic malignant tumors Our present impression is that either radioactive

phosphorus or total body irradiation with x rays given in small regularly spaced doses is far superior to urethane in the treatment of leukemias [17] When the cells become resistant to radiation therapy urethane may be worthy of a trial

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SOME OBSERVATIONS ON THE EFFECT OF FOLIC ACID ANTAGONISTS ON ACUTE LEUKEMIA AND OTHER FORMS OF INCURABLE CANCER

By Sidney Farber M D

THE PRODUCTION of temporary remissions in the course of acute leukemia in children by the administration of the compound 4 aminopteroylglutamic acid (aminopterin)[1,2]—a biologic antagonist to folic acid*—has raised a number of theoretic and practical questions *Confirmation of this finding has been reported from* several sources[3] temporary remissions equally impressive have been obtained in adults with acute leukemia by Dameshek[4]

It is the purpose of this paper to summarize briefly the status of our observations† *on the action of folic acid antagonists on acute leukemia and other incurable forms* of cancer for the interest of those now working with these agents to state the nature of some of the problems which have arisen and to indicate some directions of further research

The demonstration by Lewisohn *and his colleagues*[5] *of the occurrence of com* plete regression in about one third of single spontaneous breast cancers in three different strains of mice treated with fermentation L casei factor later shown to be pteroyltriglutamic acid (Hutchings et al [6]) and the subsequent synthesis of this *compound by SubbaRow and his co-workers*[7] *led to our study of the effect of* pteroyltriglutamic acid on incurable cancer in man Among the patients so treated were 11 children with acute leukemia The occurrence of what we called an accel eration phenomenon in the viscera and bone marrow of these patients and an experience with folic acid deficiency experimentally produced in the rat suggested that it would be worth while to ascertain if this acceleration phenomenon might be employed to advantage in the treatment of acute leukemia in children either by the use of radiation or nitrogen mustard therapy after pretreatment with folic acid or conjugates of folic acid or by the immediate use of folic acid inhibitors or

From The Children s Medical Center Boston Mass and The Department of Pathology Harvard Medical School

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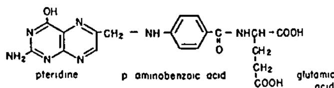
This paper is dedicated to Dr George R Minot It was my privilege when a student to hear his lec tures on diseases of the blood In these he united in masterful fashion the fields of pathology physiology and *clinical* medicine to establish a logical approach *to the nature of disease* and so *to therapy* His an nouncement when I was a fourth year student of the liver treatment of pernicious anemia fired the imagi nation of all who heard him to a consideration of the role of nutrition in other incurable diseases of unknown etiology *S F*

* By antagonist to folic acid is meant a substance which possesses the property of inhibiting the *growth of Streptococcus Faecalis R or L casei in the presence of marginal levels of folic acid Reversal of* inhibition occurs when the concentration of folic acid in the culture medium is elevated

† Our studies represent the accomplishment of a group of clinicians and laboratory workers who have joined forces to make possible rapid progress along the line indicated in this paper Detailed reports of clinical experimental toxicologic and pathologic studies are being prepared for publication

NUTRITIONALLY ACTIVE SUBSTANCES

PTEROYL GLUTAMIC ACID *(PGA, Folic Acid)*



PTEROYL DIGLUTAMIC ACID *(PG2, Diopterin)*

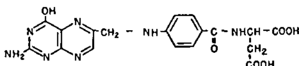
pteridine-p aminobenzoic acid two glutamic acids joined by peptide links

PTEROYL TRIGLUTAMIC ACID *(PG3, Teropterin)*

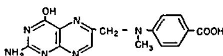
pteridine p-aminobenzoic acid three glutamic acids joined by peptide links

BIOLOGICAL ANTAGONISTS

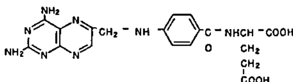
PTEROYL ASPARTIC ACID *(An Fol A or R)*



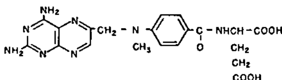
METHYL PTEROIC ACID *(Met Fol B)*



4 AMINO PTEROYL GLUTAMIC ACID *(Aminopterin)*



4 AMINO METHYL PTEROYL GLUTAMIC ACID *(A Methopterin)*



4 AMINO PTEROYL ASPARTIC ACID *(Amino An Fol)*

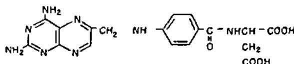


FIG 1

antagonists * The first folic acid antagonists—pteroylaspartic acid and methyl pteroic acid—were effective enough not only to give the needed encouragement for further research in this direction but also to prolong the lives of a few children with acute leukemia until more powerful antagonists of folic acid were made available The first impressive remissions in the course of acute leukemia were produced by the use of aminopterin beginning in November of 1947 These were characterized by a return almost to a normal state in some and to a state almost indistinguishable from normal in others in a group of 10 of 16 children with acute leukemia The toxicity of aminopterin emphasized the need for less toxic compounds which it was hoped might be even more effective in their carcinolytic action [1, 2]

Compounds Related to Aminopterin

Observations have been made on children with acute leukemia and on patients with a variety of other forms of incurable cancer treated by two compounds closely related to aminopterin Both of these were supplied by the late Dr Y SubbaRow These are 4 aminopteroylglutamic acid (amethopterin) and 4 aminoaspartic acid (amino-an fol) [3] A complete account of these studies will be presented elsewhere In general it may be stated that while amethopterin and amino an fol are less toxic than aminopterin exactly the same toxic changes may be produced when appropriate doses are employed This holds true for laboratory animals and for man Remissions in the course of acute leukemia in children equal to those produced by aminopterin may be brought about by the use of amethopterin or amino an fol The effective dose when remissions are obtained in children with acute leukemia lies between 3 to 5 mg a day for amethopterin and between 25–50 mg a day for amino-an fol depending upon age weight size, and physical condition of the patient These figures may be compared with a range of 0 5 mg to 1 0 mg a day of aminopterin Because there is some differential in the dose required to produce toxic changes as compared to the effective dose it has been possible to shift from one drug to another when early signs of toxicity have become apparent

Pattern of Therapy

It is impossible to present at this time a pattern of therapy as definite as that governing the use of digitalis for example or insulin Daily white count and physical examination are the best guides to the treatment to be given that day Too rapid a drop in the white count diarrhea of unknown origin the presence of stomatitis a sore tongue or ulceration of the mucous membranes of the mouth

Acknowledgement is made to the late Dr Y SubbaRow and his colleagues in the Research Division of the Lederle Laboratories (American Cyanamid Company) and their associates of the Calco Chemical Division who are responsible for the chemical research that made possible these studies on children A particular word of gratitude is expressed not only for the invaluable chemical contributions of Dr SubbaRow but also for his decision to pursue so effectively by further chemical research the leads which were obtained from these studies on children with acute leukemia The present plan of study concerning the action of folic acid antagonists is following along the lines decided with Dr SubbaRow in the spring of 1947 It consists essentially of the study of the action on laboratory animals and on patients with various forms of incurable cancer of related compounds in an attempt to find one which is more effective and less toxic than any we have previously employed

should serve as reasons for cessation of therapy until the exact cause for these disturbances has been determined In periods of remissions treatment continues as before although slightly smaller doses may be administered In some instances when patients are doing well intramuscular injection of the compound employed has been given on every other day Aminopterin apparently is effective also when given by mouth

Toxicity

Our initial report carried a warning concerning the toxic nature of aminopterin Stomatitis, ulceration of the mucous membrane of the mouth smooth tongue pharyngitis, and atrophic changes in the intestinal epithelium of the type produced by folic acid deficiency in the rat and in the monkey diarrhea gastro intestinal hemorrhage particularly when there is diffuse leukemic infiltration of the bowel and depletion of the bone marrow leading to aplasia are the most important changes *Despite efforts to prevent or to overcome quickly the toxic manifestations* by the use of liver extract various vitamin B preparations and folic acid itself in doses up to 200 mg a day for several days the most effective treatment app ars to be suspension of administration of aminopterin for four to seven days at the first sign of stomatitis or diarrhea of unexplained origin

The occurrence of hypersegmented polymorphonuclear leukocytes and the presence in the bone marrow of megaloblasts have been observed as important evidences of the effect of the antagonist It is impossible to state at this time with certainty whether all of the changes produced in acute leukemia by antagonists to folic acids are manifestations simply of a folic acid deficiency It does appear that the alterations are at the same time more profound and more subtle than those produced by folic acid deficiency alone and that interference with biochemical systems more important than simple competitive substitution of the antagonist for folic acid *within cells must obtain Evidence bearing on this point is being collected*

Hemorrhage

Hemorrhage into the gastro intestinal tract the skin and the genito urinary tract and the cranial vault either massive or oozing in character has always been one of the most serious complications of acute leukemia and one of the important causes of death Studies now being conducted by our group following the work of Allen and Jacobsen[9] show that in many children with acute leukemia the level of heparin like substances in the blood is definitely higher than the normal While bleeding occurs usually when the level of blood platelets is low thrombo ytop nia *may be present without any evidence of ble-ding for many months* The longer survival of patients with acute leukemia made possible by folic acid antagonist therapy has brought the problem of hemorrhage into great prominence The combination of leukemic infiltration of the intestinal tract and toxic effects produced by aminopterin amethopterin and amino an fol makes for the ready occurren e of gastro intestinal hemorrhage Although the exact explanation is not cl ar it appears certain that hemorrhage occurs more readily if the bone marrow is markedly depressed by *the compound employed The effect may be similar to that pro*

duced in aplastic anemia where gastro intestinal hemorrhage is a common and serious occurrence If toxic levels of the folic acid antagonist are employed long enough the bone marrow may be depressed enough to accentuate the hemorrhagic tendency in leukemia or to act as the sole cause of the hemorrhage

Nature of Leukemia

Observations on a girl (M D) 8 1/12 years old at the time of her death and similar experiences with other children have raised a question concerning present conceptions of leukemia This child lived for twenty two months after the onset of acute leukemia Treatment with pteroylaspartic and methylpteroic acid was followed by repeated temporary periods of improvement She died following uncontrollable oozing from the mucous membranes Postmortem examination revealed leukemic cells so few in number in scattered areas throughout the body that the diagnosis of acute leukemia would have been made with hesitation on the basis of that evidence alone It seems probable that hemorrhage in acute leukemia may be produced by a number of different factors apart from the effect of leukemic infiltrates on the bone marrow and viscera and the thrombocytopenia The hypothesis seems warranted that a serious disturbance in the hematopoietic system or a series of deficiencies in the body responsible for oozing or for massive hemorrhage might still be present in the patient with acute leukemia if every leukemic cell in the body could be destroyed Acute leukemia, therefore may be a form of cancer complicated by specific deficiency states—a suggestion that has definite implications for further research

Types of Leukemia

In the majority of the children with acute leukemia treated it was impossible to diagnose with certainty the exact morphologic type of leukemia because of the primitive nature of the blasts It would seem logical and certainly highly desirable to replace or to supplement the morphologic classification of leukemia by one based upon response to specific stimuli such as the folic acid antagonists Study of those patients with acute leukemia who failed to respond to these compounds might yield data of value concerning the nature of the disease A worthy goal is the characterization of the various types of acute leukemia in terms of precise intracellular biochemical deficiencies or alterations

Results

In a group of approximately 60 children with acute leukemia treated for three weeks or longer with either aminopterin amethopterin or amino an fol somewhat more than 50 per cent showed improvement clinically hematologically of important degree attributable to the action of these compounds Detailed tabulations of our entire experience with thorough documentation will appear separately Two of the five children where case histories were presented in our initial report are still alive (December 21 1948) Case 1 of that report a boy of 8 has a history of acute leukemia beginning in February 1947 He was treated first with methyl pteroic acid and pteroylaspartic acid Aminopterin was not given until Decemb r

16 1947 Since then that or one of the other more powerful folic acid antagonists have been employed Leukemia is still present and there have been many complications but he is still alive twenty three months after the onset of his disease A second child mentioned in the earlier report Case 5 has had acute leukemia since August 1947 He is one of twins and despite his leukemia and almost constant folic acid therapy he is as tall and as well nourished as his brother His leukemia which is still recognizable by studies of bone marrow and peripheral blood is still under control sixteen months after onset

The widespread use today of aminopterin in the treatment of acute leukemia has raised for discussion a basis of comparison of results Any evaluation of treatment of patients with incurable cancer must rest upon a solid foundation of knowledge concerning the life history and biologic behavior of tumors Acute leukemia which runs an invariably fatal course varying from a few weeks usually to six months after onset of symptoms lends itself readily to comparative studies Rarely the course may last as long as twelve months and isolated instances of longer survivals have been observed The end point of time itself therefore should serve as a reliable criterion of the value of any form of therapy

Spontaneous remissions either complete or partial occurred in 10 per cent of 300 children with acute leukemia observed by Dr Louis K Diamond [10] at the Boston Children s Hospital These averaged slightly less than ten weeks in duration In two instances a second remission was observed In almost 75 per cent of these children in whom spontaneous remission was noted there was a history of infection of important degree immediately preceding the remission The recent production of remission in acute leukemia by the use of massive blood transfusion makes necessary the evaluation of this factor too in patients treated with folic acid antagonists Analysis of our experience permits the statement that the remissions we have described are dependent neither upon infection nor transfusions of blood

It is obvious that no two children with acute leukemia present strictly comparable problems Infiltration of the leukemic processes is generalized but there are great variations in the degree and site of involvement In one a large subdural accumulation of tumor may alter intracranial pressures to an important degree in another the leukemic infiltration in the heart may be responsible for unexpected death Other variables are the amount of replacement of the bone marrow by leukemic cells the factors responsible for bleeding and the occurrence of secondary infections It should not be surprising therefore if one research group reports five consecutive remissions (personal communication from Dr George Guest Cincinnati Children s Hospital) or that another group observes a fatal outcome within two weeks after onset of therapy in ten consecutive patients before one remission is observed The arbitrary limit of three weeks after onset of therapy has been chosen for a basis of comparison During this period those patients most severely involved will have died or the folic acid antagonists employed will have had an opportunity to effect the tumor infiltrations in the viscera and the bone marrow

It should be emphasized that all available resources of medicine have been utilized in an attempt to prolong the lives of our patients with acute leukemia Transfusions radiation therapy antibiotics and specialized dietary measures have all been

employed when indicated It has been possible however to study a sufficient number of patients for long enough periods of time with folic acid therapy alone to permit the accumulation of sufficient data upon which reliable conclusions could be based It should be expected therefore that considerable variation in the results of different investigators will be reported until a sufficiently large experience has been obtained or until a long enough period has elapsed to permit the use of the period of survival alone as the simplest criterion of therapeutic effect

The effect of these folic acid antagonists despite some theoretic considerations which entered into the formulation of early working hypotheses is not limited to acute leukemia We have reported[11] temporary definite but inconstant carcinolytic action on patients with apparently unrelated forms of incurable cancer such as neuroblastoma and pulmonary metastases from cancer of the bladder as well as more closely related tumors such as lymphosarcoma and Hodgkin s disease

The range of carcinolytic action on various types of incurable cancer in man is now being evaluated The combined action of the folic acid antagonists when employed with other agents used in the treatment of cancer such as the sex hormones and radiation therapy is under study

The toxic nature of the compounds employed in these studies and the inconstant and temporary nature of beneficial effects make clear that the value of these compounds is still limited to research The finding of equally or more effective and less toxic compounds and an understanding of the reasons for failure in those patients who do not respond are goals which must be reached before more widespread application of the results of these studies is possible

Summary

A general discussion is presented of the present status of folic acid antagonist therapy in acute leukemia in children and in other forms of incurable cancer Conclusions reached in our initial report have been supported by a far greater experience Temporary remissions in acute leukemia as marked as those caused by aminopterin have been produced by the use of two compounds closely related chemically to aminopterin —amethopterin and amino an fol both of which however are also toxic compounds Despite the increasing number of patients in whom temporary remissions have been produced with survival in some far beyond the usual course of the disease no evidence has been presented which would justify the use of the word cure of acute leukemia A carcinolytic action on related and on certain unrelated forms of incurable cancer has been observed Further research for less toxic related compounds with even greater effectiveness is not only justified by these studies but is imperative The value of this direction of research in cancer has been established

Two of the most pressing problems demanding solution are concerned with the nature the prevention and the treatment of toxic changes including hemorrhage produced by these folic acid antagonists and the causes prevention and mechanism of hemorrhage in acute leukemia The use of the folic acid antagonists in the treatment of incurable cancer including leukemia must remain in the realm of research until answers to these questions are found

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ANTIBIOTICS IN CHRONIC LEUKEMIA

By DUDLEY MERRILL M D

THE day by day care of patients with chronic leukemia of any type is one which will tax the ingenuity of the physician to the utmost These patients can sometimes be carried along for several if not many years in comparative comfort with X ray therapy and transfusions as often as necessary to maintain a hemoglobin between twelve and thirteen grams One of the characteristics of these patients is their liability to infections and their extremely poor resistance to them In fact infection is one of the common causes of death in this particular group

Prior to the discovery of the sulfonamides penicillin streptomycin and tyrothricin an attack of pneumonia a cellulitis of the throat or an infected hematoma often resulted in death in a few days from overwhelming sepsis This was true even in patients whose general condition was such prior to the infection that many weeks or months of active life could have been anticipated in spite of the leukemia

For many years it was seriously questioned by many authors whether X ray therapy actually prolonged life in patients with chronic leukemia It is practically impossible to prove this proposition statistically because of the extreme variation of life expectancy in these patients without any treatment whatever When life expectancy ranges from six months to twelve years an average or a mean becomes a mathematical figure of little or no significance when applied to an individual case

The same is true of any attempt to evaluate in a statistical fashion the effect of antibiotics nitrogen mustards urethane or Fowler s solution on the course of leukemia

The extraordinary value of the antibiotics in the management of infections in general has now been conclusively established These drugs have also revolutionized many of our long accepted medical and surgical beliefs as well as many of our apparently well established rules of prognosis

The care as well as the prognosis of patients with pneumonia meningococcus meningitis spreading streptococcal infection osteomyelitis and bacterial endocarditis have been profoundly altered in the past ten years

One of the most revolutionary technics for the handling of mild or severe cellulitis and lymphangitis localized or spreading including even the face and nose has been the injection of penicillin in large doses directly into and around the infected tissues Rose and Hurwitz[1] have demorstrated the complete safety and great usefulness of this method The superiority of this approach over parenterally administered penicillin at a distant site is not surprising when one considers the immense concentrations of penicillin achieved at the site where it is most needed Comparable concentrations from penicillin injected at a distance from the infection either intramuscularly or intravenously obviously would necessitate massive doses The simplicity and inexpensiveness of the procedure are also in its favor

Luckily none of the effectiveness of the antibiotics depends upon the integrity

From the Mt Auburn Hospital Cambridge Massachusetts

of the leukocytes Thus they are equally efficacious in coping with infection in patients with profound disorders of the white blood cells such as agranulocytosis aplastic anemia or any form of leukemia Two cases* are presented to illustrate

Case Reports

Case No 1 J J This 15 year old boy was diagnosed as having leukemia after having been run down for about four months His blood count showed Hgb 34 per cent (S) RBC 1 69 and WBC 25 600 The smear showed 60 per cent large immature white cells These were classified as either immature monocytes or histiocytes by a consulting hematologist Treatment was decided upon in spite of the obviously poor prognosis He was repeatedly transfused and given small doses of x ray therapy with rapid symptomatic improvement

About a month after treatment was started he developed a painful hematoma in his right calf and smaller ones in the sole of his left foot and in his left cheek In the next week his temperature rose gradually to 105 F in spite of full doses of sulfadiazine by mouth The hematoma of his leg increased in size induration redness and painfulness until it occupied an area 15 x 10 cm It finally broke down and discharged vast quantities of serosanguineous material Coping with such a large ulcer which oozed such enormous quantities of serum blood and pus and was extremely painful when dressings were changed was finally solved by Doctor Robert Linton who placed the leg in a cast and packed the ulcer with gauze impregnated with tyrothricin ointment There was a dramatic improvement in the boy s general condition with a drop of his temperature to normal In three days time he was able to be up in a wheel chair When the cast was changed after thirteen days the ulcers were filling in rapidly Eighteen days later he was allowed home where he was comfortable getting about in a wheel chair for the next month except for one brief return to the hospital for further transfusions Finally however he had a recurrence of multiple hematomas his white count rose to 100 000 with 98 per cent extremely immature cells and he went downhill fairly rapidly in spite of x ray therapy transfusions and tyrothricin applications to his ulcerated hematomas He died approximately five months after the diagnosis was established and eight months after his first symptoms

Postmortem examination established the diagnosis of monocytic leukemia

Case No 2 Mrs M M This 74 year old woman was first seen because of anemia She had a Hgb 46 per cent (S) RBC 1 90 WBC 3 250 The differential showed P 44 L 32 M 24 and 2 nucleated RBC/100 WBC The platelets were normal the RBC deeply stained and there were many tailed cells It was felt that her blood picture was consistent with pernicious anemia

After one month on liver extract 15 units intramuscularly once a week her Hgb was 35 per cent (S) RBC 1 55 and WBC 4 500

A sternal biopsy was then done and reported by Doctor H E MacMahon as follows The overall picture is one of aplastic anemia but there are tiny foci showing a picture consistent with pernicious anemia under treatment

Large doses of folic acid liver extract powder and intramuscular liver extract were given but there was no response whatever The patient was kept in excellent health without any complaints for a whole year by repeated blood transfusions

Then increasing numbers of abnormal white cells began to appear coincident with the development of areas of cellulitis on both sides of her neck and in both nostrils The next day penicillin 25 000 units every three hours intramuscularly was started but in two days time the cellulitis had spread and now involved the lower lip and cheek which were enormously swollen She was then given 50 000 units of penicillin in 5 cc of saline directly into the infected areas There was rapid improvement with resolution of the areas of cellulitis without any breaking down of the tissues Parenteral penicillin was stopped *nine* days after the local injection had been given

Six days later however a new area of cellulitis on the left side of the lower lip had appeared and paren

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* Both of these cases are reported through the courtesy of Dr Arthur N McKechnie of Cambridge Mass

teral penicillin every three hours was again started Five days later this was changed to single daily injections of 300 000 units of penicillin in peanut oil and beeswax because of breaking down and abscess formation in the areas previously injected with penicillin in saline

On this treatment the areas of cellulitis about the lips and mouth subsided but she developed many new areas about the chin cheeks neck right upper arm and both buttocks Her white count rose to 64 600 with more than 50 per cent extremely immature forms

All therapy was finally abandoned and the patient died about ten weeks after the first appearance of the areas of cellulitis in her neck She had received a total of 19 575 cc of whole blood in the course of fourteen months and for twelve of those months she had had extraordinarily few if any complaints

Autopsy showed monocytic leukemia and extensive hemosiderosis of liver spleen lymph nodes and bone marrow consistent with hemochromatosis This latter finding was presumably due to her enormous number of transfusions

Conclusion

The new antibiotic drugs sulfonamides penicillin, streptomycin and tyrothrycin have proved useful in the management of the infectious complications in leukemia and other disorders of the bone marrow

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MONOCYTIC LEUKEMIA

By Carl J Gessler M D

THE publication of detailed case reports may often be of value A case of monocytic leukemia is reported here in detail, in the hope that it may throw some light on our knowledge of this condition

Report of Case

The patient was a 36 year old married white woman who lived in Brussels When first seen on August 8 1946 her chief complaint was that of progressive weakness of several months duration The most interesting feature of the past history was a seri s of skin symptoms In January 1946 an itching erythema appeared on the inner aspect of both thighs and one month later the forearms and neck became similarly affected These manifestations disappeared about the middle of March The patient stated that for about eight days at the beginning of July she had had some kind of eczema on the neck A few days later on July 18 numerous brownish red slightly itching papules had appeared on the trunk and had persisted for about a week

The patient was very pale and her face was swollen The spleen was not palpable no lymph glands were felt the liver was tender and felt 2 cm below the co tal margin in the mid clavicular line Blood laboratory examinations revealed the following Hemoglobin 28 per cent = 4 37 Gm per 100 cc red blood cell count 2 22 million per cu mm color index 0 6 total white blood cell count 50 500 per cu mm Examination of the stained smear (May Grunwald Giemsa) revealed the presence of numerous large cells with irregular nuclei The differential count (based on 400 white cells) was as follows

20 25% neutrophils (9 25% of them staff cells)
0 % eosinophil
0 % basophil
12 75% lymphocytes
56 % monocytic cells of abnormal morphology
9 5 % paramyeloblasts
65 5 leukemic cells
0 5 % neutrophil metamyelocytes
1 % neutrophil myelocytes
3 normoblasts were found per 400 leukocytes

Aspiration of the bone marrow by sternal puncture proved unusually difficult finally however small lumps of whitish material were obtained practically free from blood The smear of the specimen was almost entirely made up of leukemic cells which could be classified as pathologic promyelocytes or promyelocytoid paramyeloblasts *Not one cell was s en in the sternal puncture p eparation which was comparable with the monocytic type of cells found in the blood*

The patient was seen again three we ks later and at that time her condition had deteriorated rapidly Considerable edema of the face extreme weakness and extensive ulcero necrotic lesions of the mouth were noticed The temperature as 39 C (102 2 F) A few bro n macules half a centimeter in diameter remained from an extensive eruption of red spots hich had appeared during the interval between the two examinations The blood pressure was 120 40 Blood examination sho ved the following Hemoglobin 11 per cent = 1 72 Gm per 100 cc red blood cells 1 04 million per cu mm and leukocytes 184 600 per cu mm The differential count as as follows

3 25% neutrophils (1 staff cell)
0 % eosinophil
0 % basophil
4 25% lymphocytes
77 5 % monocytic cells of abnormal morphology 92 leukemic cells

14 5 % paramyeloblasts
0 5 % plasmacyte
2 normoblasts were found per 400 leukocytes

The patient died on August 31

Discussion

The ulcero necrotic lesions of the mouth were perhap particularly significant Forkner[1] stated that such lesions are more constant and extensive in monocytic leukemia than in other varieties of leukemia The cutaneous manifestations are also worthy of note Some of them were atypical and probably allergic in origin



Fig 1 Blood Smear

while others consisting of brown or red maculo papulae were more or less characteristic of the reticulo-endothelioses

There seems to be no possible doubt as to the diagnosis of leukemia However opinions could differ as to the exact classification A diagnosis of monocytic leukemia *was substantiated by the high percentage of abnormal monocytic cells* in the circulating blood These cells are extremely polymorphous and can be divided into two main groups although such divisions are always somewhat arbitrary (1) The majority were large cells containing irregular nuclei without nucleoli the pale blue protoplasm being entirely filled with a gr at number of very small azurophil granules which are characteristic of the monocyte (2) Other cells very similar to the previous ones had younger nuclei containing nucleoli and the fine azurophil granulation occupied only part of the protoplasm sometimes being confined to the perinuclear zone It is on the basis of these criteria that Osgood distinguishes monocytes and promonocytes In the first differential count in a

total of 56 monocytic cells 50 belong to the first group and 6 to the second in the second differential count on a total of 77 5 monocytic cells 46 5 belong to the

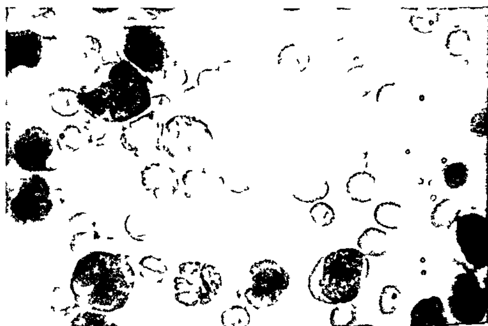


Fig 2 Blood Smear

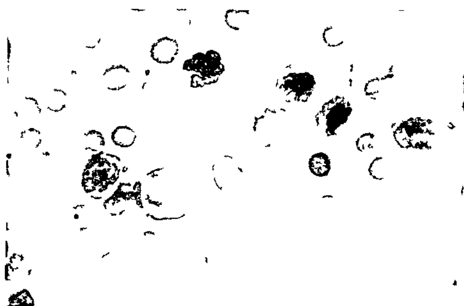


Fig 3 Blood Smear

first group and 31 to the second which shows an increase of the more immature cells

However as mentioned before there was a marked discrepancy between the

blood smear and the marrow smear Considering only the marrow smears one would have no hesitation in making a diagnosis of myeloid leukemia The case could then be interpreted in two different ways (1) If one believes that the monocytic cells of the blood are derived from the leukemic cells in the marrow the case could be

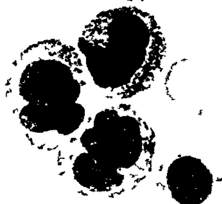


FIG 4 BLOOD SMEAR



FIG 5 MONOCYTIC CELL WITH PSEUDOPODA
(Note granule extending within the pseudopode)

termed *paramyeloblastic leukemia* (2) When one assumes the monocytic cells of the blood *are not derived from the leukemic cells in the marrow but express a reaction* of the reticulo-endothelial system elsewhere in a patient with myeloid leukemia which Oberling[3] has called reticuloses associees Oberling has given instances of this association

In our opinion, it would be an overextension of the concept of the paramyeloblast to consider, as such, the monocytic cells in the peripheral blood of our case

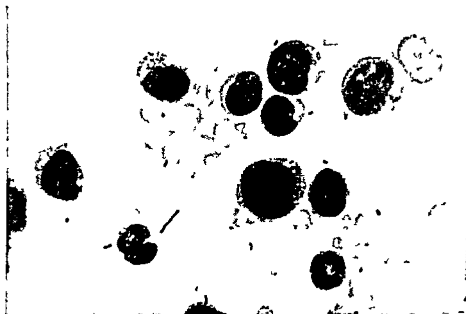


Fig 6 Marrow Smear



Fig Marrow Smear

On the other hand their morphology seems too definitely abnormal to regard them simply as a result of a simple reaction of the reticulo endothelial system Despite the lack of monocytes in the bone marrow it was felt that there was strong evi

dence for a diagnosis of monocytic leukemia which can be defined further according to the currently prevailing classification as belonging to the Naegeli type (characterized by leukemic proliferation in the bone marrow) as opposed to the Schilling type (or leukemic reticulosis) characterized by proliferation of the reticulo endothelial system elsewhere than in the bone marrow

Author n t

It is interesting that the patient s brother died in 1942 at about the same age (35) of a mediastinal tumor Unfortunately no answer was received to our inquiries concerning the nature of this tumor which might well ha e been a localiz d growth of reticulum cells

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FOLLICULAR LYMPHOBLASTOMA

A Report of Six Cases

By Ovid O Meyer, M D

FOLLICULAR lymphoblastoma has been a subject of considerable interest since 1925 when Brill Baehr and Rosenthal[1] reported the 2 cases which made the medical profession really cognizant of this entity They called the condition generalized giant lymph follicle hyperplasia of lymph nodes and spleen Much earlier in 1901, Becker had described a case which probably was this disease and between 1912 and 1920 descriptions[3-5] of a few additional cases were reported In their original report Brill Baehr and Rosenthal[1] concluded that the lymph node disease was probably benign but in a subsequent report published two years later Baehr and Rosenthal[6] concluded on the basis of six cases studied that the condition was malignant In the same year however Symmers[7] described 3 cases on the basis of which he concluded that the condition was benign from the standpoint of prognosis In an extensive report published in 1938 Symmers[8] described with the histologic picture cases of giant follicle hyperplasia which later were transformed into polymorphous cell sarcoma of the lymph follicles others which appeared to terminate as Hodgkin s disease and still others which ended as lymphatic leukemia He stated that giant follicular lymphadenopathy with or without splenomegaly was probably inflammatory or toxic in origin and usually amenable to mild roentgen therapy In a communication published in 1946 [9] he stated that he had seen 5 histologically proved cases in which roentgen therapy was followed by apparent cures for periods of 3 4½ 5 9[1] and 12 years Another patient survived nine years without any treatment Baggenstoss and Heck [10] on the other hand agree with the conclusion which Baehr Klemperer and Rosenthal[11] had arrived at by 1931 namely that the disease is a form of lymphosarcoma which in its early stages presents the histologic picture of follicular hyperplasia but is later characterized by a conglomeration of the follicles and diffuse infiltration of the lymph nodes by polymorphous lymphoblasts It was at this time that the term follicular lymphoblastoma was proposed [11] By 1940 Baggenstoss and Heck[10] had collected 59 cases from the literature and 13 of their own a total of 72 In 1941 Gall Morrison and Scott[12] reviewed 63 cases from biopsy or necropsy material submitted to the laboratory of the Massachusetts General Hospital Since publication of this report more than 50 additional cases[13-29] have been recorded through 1946 These include 15 cases with skin manifestations reported by Combes and Bluefarb [30] The present paper reports 6 additional cases

The chief characteristics of follicular lymphoblastoma are outlined by Baehr Klemperer and Rosenthal [11] Enlargement of the lymph nodes is due to enormously enlarged lymph follicles a single one of which may fill a low power microscopic field The follicles resemble huge germinal centers consisting of lymphoblasts with

From the Department of Medicine University of Wisconsin Medical School

frequent mitotic figures The periphery of each large follicle is surrounded by a narrow zone of small lymphocytes whose nuclei stain darker The splenomegaly may be enormous as a result chiefly of enlargement of Malpighian bodies There are no abnormal cells in the blood Nor is anemia and cachexia present until the end stages of the disease There is a tendency to lymphatic infiltration in the lacrimal gland which gives rise to unilateral exophthalmus and there is a tendency to involvement of serous membrane with pleural or peritoneal serous or even chylous effusion The disease is remarkable for its chronicity and its extreme radiosensitivity No neoplastic disease responds more promptly to relatively small doses of roentgen or radium therapy Recurrences in widely separated parts of the body usually take place after varying intervals until eventually often after many years there is radioresistance

The present report is of 6 cases seen at the State of Wisconsin General Hospital since 1941 Two cases 2 and 6 were of particular interest because of bone involvement Three of the patients were women 3 were men The ages were 54 45 44 61 82 and 60 respectively

Case Reports

Case 1 L M M age 54 a white farm housewife was admitted September 3 1941 She complained that for six months she had been afflicted with shortness of breath and a cough In the spring of 1941 she noted a painless swelling of the right side of the neck She saw a physician on July 3 and was hospitalized A left pleural effusion was demonstrated and thoracentesis was performed on three occasions each of which was followed by temporary relief of dyspnea

Examination showed an obese white woman able to sit up in bed The trachea was deviated to the right There were 2 cm nodes in the right cervical region several 1 cm nodes in the left posterior cervical chain and a healed surgical scar of biopsy on the right The nodes were freely movable nontender and without induration No other enlargement of lymph nodes was demonstrated There were signs of a massive fluid accumulation in the left thorax The blood pressure was 110/90 The liver extended 3 cm the spleen 8 cm below the costal margin

Laboratory studies showed a hemoglobin of 14 5 Gm (90 per cent) erythrocytes 3 930 000 leukocytes 5 500 neutrophiles 64 lymphocytes 32 per cent monocytes 2 eosinophiles 2 per cent Blood Wassermann was negative A roentgenogram of the chest as read by Dr L W Paul showed massive left sided opacity presumably due to pleural effusion Bucky film after aspiration of fluid did not demonstrate enlargement of mediastinal nodes The tuberculin test was negative

Thoracentesis on three successive days resulted in the withdrawal of 1500 cc of chylous fluid on each occasion No organisms were found in the fluid and it was negative for acid fast bacilli The specific gravity was 1 023 and albumen 2 per cent there were many red blood cells Biopsy of a node from the right cervical region on September 6 1941 showed the findings of follicular lymphoblastoma

The patient received eight treatments of 150-r to 200-r each in air (H V L = 1 05 mm distance 50 cm) between September 8 and 13 1941 and she went home In October she returned to the outpatient department Earlier in the month she had required a thoracentesis There had been no gain in weight but she felt better Her cough however and the signs of a small fluid accumulation in the left pleural cavity persisted There were no palpable lymph nodes the spleen was about 6 cm below the costal margin On January 8 1942 the patient reported a ravenous appetite an 8 pound gain in weight and for three weeks recurrent backache in the mid lumbar region which was severe enough to cause much loss of sleep The cough had disappeared Examination showed tiny cervical and axillary nodes The spleen was 2–3 cm below the costal margin There was muscle spasm and tenderness in the right lumbar region A roentgenogram of the lumbar spine was negative for bone or joint disease Three treatments of 150-r each were given to the spine Subsequently after the patient had returned home massage and diathermy were administered since the pain persisted

She was readmitted to the hospital on April 27 1942 suffering torturing pain in the back and down into the legs She had increasing difficulty in walking and loss of control of the bladder and bowels

Physical examination at this time revealed signs of a moderate accumulation of fluid in the left pleural cavity the spleen was just palpable and there was no enlargement of the lymph nodes The patient could no longer move the lower extremities the deep reflexes had ceased and the vibratory sense and position sense were absent There appeared to be deformity of the 8th thoracic vertebra

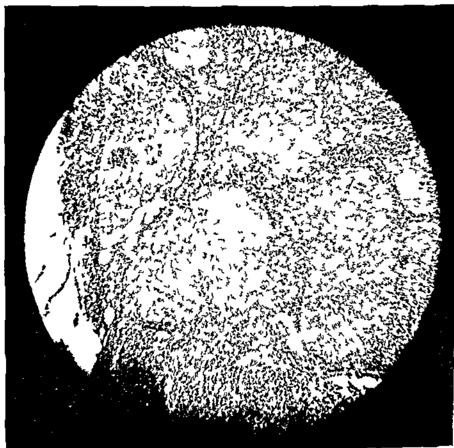


FIG 1 SECTION OF A CERVICAL LYMPH NODE OF PATIENT L M M CASE NO 1 MAGNIFICATION 30X

Laboratory studies showed the hemoglobin to be 11 4 Gm (70 per cent) leukocytes 4 500 with 76 per cent neutrophiles A roentgenogram of the dorsal and lumbar spine showed no definite bone lesion

It was believed that the patient had a lesion compressing the cord and five treatments totaling 1100-r in air (H V L = 1 05 mm distance 50 cm) were administered to the dorsal and lumbar regions She returned to the hospital for the last time on July 9 1942 Her condition has not improved ten days before her legs had become slightly flexed at the hips and knees and it was almost impossible to move them

There was anesthesia in both legs due to apparent sacral segment lesion and in the right leg it extended to lumbar 4 Deep reflexes were absent Roentgenograms of the dorsal and lumbar spine were negative The laboratory studies were not significantly different from those made in April Six treatments of 200-r each in air (H V L = 2 4 mm distance 60 cm) were administered to the dorso lumbar spine between July 1 and 7 1942 and the patient was discharged She died at home about two months later and autopsy was not obtained

Comment Involvement of the pleura with effusion was prominent in this patient The unproved but apparent involvement of the spinal cord was unusual The course was rapid death occurring within 18 months of onset of the lymph node enlargement

Case 2 C A J age 45 an American housewife of Dutch descent was admitted on May 13 1946 complaining of pain in the left leg Her illness had begun three years before with episodes of gaseous distension nausea and diarrhea Several months later she noted enlargement of nodes in the inguinal regions enlargement of the abdomen and then the presence of lumps in the neck and axillae Biopsy was made and x ray therapy given The nodes disappeared A few months later she complained of pains in the arms legs and lower abdomen These gradually decreased without specific therapy After this she had been able to do hard work until two months before admission when she again suffered pain in the left leg and lower back

Physical examination showed a well-developed well-nourished though pallid woman The largest of a few palpable nodes 2 cm in diameter was in the right axilla The liver and spleen were not palpable

Laboratory studies showed 4 plus glucose in the urine hemoglobin 11 Gm (70 per cent) erythrocytes 3 413 000 leukocytes 2 700 with neutrophiles 69 eosinophiles 0 6 small lymphocytes 7 atypical lymphocytes 2 6 intermediate and large lymphocytes 3 4 young lymphocytes 5 monocytes 11 4 metamyelocytes 0 8 neutrophilic myelocytes 0 2 per cent Reticulocytes numbered 1 4 per cent The volume of the individual cell was 94 2 cu microns A roentgenogram of the chest showed no enlargment of mediastinal nodes The roentgenogram of the pelvis as read by Dr L W Paul showed areas of increased density in the ischial rami particularly on the left side with a suggestion of a little mottling in the upper ends of the femurs These changes were thought by Dr Paul to be consistent with early involvement of these bones by a lymphoblastoma

Biopsy of an axillary lymph node was performed the description of Dr Walter Jaeschke being as follows This moderately enlarged node has a moist grey medullary appearance on cut section Throughout there are giant follicles separated by a lymphoid stroma In the stroma there are numerous eosinophiles Reed Sternberg cells are not identified Diagnosis giant follicle lymphoblastoma

The patient received 600 r in air of high voltage roentgen therapy (H V L cu = 1.05 mm distance 50 cm) to the right and left axilla and a similar dosage to the anterior and posterior pelvis She was discharged on May 22 1946 Her local physician reports that the patient died in July 1946

Comment This patient who had symptoms for three years before coming under our observation had definite anemia by this time There is in the literature little to suggest involvement of bone with follicular lymphoblastoma but since it is common in other types of lymphoblastoma its occurrence in 2 of the 6 cases here described is probably not surprising

Case 3 E R H a 44 year-old white male was first admitted to the Wisconsin General Hospital on April 28 1942 complaining chiefly of lumps on the head and left side of the neck He had had an enlarged node behind the right ear as long as he could remember About nine months before admission he had first noted three small lumps on the right side of the head These slowly increased in size and a week before admission another lump appeared on the left side of the head A biopsy of a node from the scalp showed hyperplastic follicles He had lost 22 pounds in weight

Physical examination showed this patient to be a well-developed well nourished man of good color On the right side of the head were three firm but movable masses measuring 2 x 3 to 2 x 4 cm On the left side also in the region of the scalp was a single smaller node and in the left submaxillary region was a firm node measuring 3 x 5 cm In the neck were several smaller nodes and there were also small nodes in the right postauricular the axillary epitrochlear and inguinal regions Blood pressure was 200 systolic 120 diastolic The liver extended 3 cm the spleen 2 cm below the costal margin on deep inspiration

Laboratory studies showed a hemoglobin of 15 9 Gm (100 per cent) erythrocytes 4 280 000 leukocytes 8 600 with neutrophiles 76 per cent lymphocytes 23 per cent eosinophiles 1 per cent Blood

Wassermann was negative The basal metabolic rate was plus 37 Roentgenograms of the chest showed cardiac enlargement X ray of the skull was negative Biopsy of the node in the left submandibular region was interpreted by Dr W D Stovall as giant folliculoma (follicular lymphoblastoma)



FIG 2 SECTION OF AN AXILLARY LYMPH NODE CASE NO 2 MAGNIFICATION 60X

The patient was discharged on May 5 1942 to report to the outpatient department for roentgen therapy He has been seen repeatedly in the outpatient department and has twice been readmitted to the hospital in May 1943 and on January 11 1945 at which time he complained of dyspnea orthopnea precordial pain and cardiac arrhythmia For five months he had been hoarse and for a week had noted enlargement of the spleen with pain in this region The right side of the neck was swollen

The patient was apprehensive There was general superficial enlargement of the lymph nodes several small nodules scattered in the subcutaneous tissue signs of bilateral intrapleural fluid The liver extended 6–8 cm below the costal margin and the spleen was palpated 16 cm below the costal margin The blood pressure was 185 systolic 120 diastolic

Laboratory stud es included a urinalysis with 5 to 10 casts per lo power field specific gravity 1 0 6 The blood count was still essentially normal A roentgenogram of the abdomen sho ed enlargement of the spleen and liver An x ray of the chest showed the presence of fluid bilaterally There was al o moderate enlargement of the mediastinal nodes

It was thought that much of the symptomatology and the pleural effusion were attributable to the cardiac disea e rather than to the lymphoblastoma However the patient received 12 treatments of 150-r each in air (H V L cu = 1 05 mm distance 50 cm) distributed to the spleen right cervical and mediastinal regions He as discharged January 25 1945 *On April 15 of that year his physician reports the* patient d ed of cardiovascular renal syndrome

Comment The duration of this case of lymphoblastoma could not be ascertained but seemingly it was long if the original lumps are significant If not then the course was short death resulting from the cardiac and renal failure induced by the severe and persistent hypertension Pleural effusion is common in follicular lymphoblastoma but here it was thought to be cardiac rather than lymphomatous in origin

Case 4 Mrs J H *age 61 the wife of a missionary to China for many years visited the outpatient* department on March 5 1946 with the complaint of a lump in the right groin Sixteen years before hile in China her left eye had been remo ed for what as found to be a malignant tumor of unknown type On July 24 1941 32 cm of rectum and sigmoid were removed and a colostomy performed for a neoplasm which was localized and found to be an adenocarcinoma On May 17 1943 a tumor in the left inguinal region was removed A microscopic study of this lymph node showed greatly enlarged lymph follicles with some confluence The individual cells making up these follicles possessed a curious pleomorphism They invaded the capsule and the surrounding fat The diagnosis vas malignant lymphoblastoma A preauricular lymph node which had be ome enlarged in 1943 disappeared completely follo ing high voltage roentgen therapy Late in February 1945 a growth on the hard palate was resected Sections showed fibrous tissue moderately infiltrated with lymphocytes a few plasma cell eosinophiles and occa onal polynuclear neutrophiles There ere no Reed Sternberg cells The interpretation as chronic granulomatous inflammation On April 3 1945 a tumor was remo ed from the region of the right scalenus muscle sections as studied by Dr S B Pessin sho ed a distorted lymph gland vith the normal architecture completely destroyed The predominant cell were large and medi m sized lymphocytes containing a vesicular nucleus ith one or t vo distinct nucleoli There was a small amount of fibrosis in some areas and considerable delicate reticulum Occasional mitotic figures ere seen The d agnosis was reticulum cell lymphosarcoma

Early in November 1945 *the patient noted a lump in the right groin* h ch brought her to the State of Wisconsin General Ho pital on March 6 1946 Eight days later the node from the right groin was removed and at the same time a 3 cm node from the thyroid was excised Gene al examination at this time showed no other lymph node enlargement nor enlargement of the spleen and liver There was no anemia the total leukocyte count as 4 300 ith a normal differential count The section from the thyroid showed clo ely spaced giant follicles but some fusion of follicles had taken place so that the picture resembled a fully developed reticulum cell lymphosarcoma The diagnosis of Dr Walter Jaeschke as follicular lymphoblastoma

The patient has remained ell to date and her case has been followed in the outpatient depa tment

Comment This is a rather amazing case with the evidence quite reasonably substantiated of multiple malignancies The reports of the several lymph node studies might well leave one confused and perhaps the pathological findings were

not the same in the original studies as in the last * This emphasizes it seems the close relationship between follicular lymphoblastoma and other malignant disease (lymphosarcoma) The last biopsy was sufficiently characteristic to justify the inclusion of the case in this series

Case 5 *J G* This patient a white male of 82 was admitted to the State of Wisconsin General Hospital on April 24 1945 He had an enlarged right tonsil which had first been noticeable four months before and had been progressively growing There was no pain nor bleeding and although the patient was conscious of the mass it caused no real difficulty in eating

The patient was very deaf and almost blind The teeth were very carious The enlarged right tonsil protruded well into the midline and filled half the throat The mass was irregular hard and nontender There was no enlargement of the lymph nodes nor of the spleen and liver

The blood count showed a hemoglobin of 11 6 Gm (70 per cent) erythrocytes 3 600 000 the leukocyte count was normal A roentgenogram of the chest showed no enlargement of mediastinal nodes

The tonsils were removed surgically The left was fibrous The right tonsil was 4 x 2 5 x 2 cm There was loss of usual architecture although a number of large giant follicles could faintly be made out Throughout the sections there were occasional endothelial cells numerous small round cells closely resembling lymphocytes and occasional mitotic figures The interpretation of Dr W D Stovall was follicular lymphoblastoma The patient died of bronchopneumonia six days after the operation Post mortem examination was not permitted

Comment The disease in this instance involved so far as could be determined only the tonsil Baehr Klemperer and Rosenthal[11] observed that in their cases the tonsils and lymphatic apparatus of the gastrointestinal tract had not been involved Baggenstoss and Heck[10] report 2 cases with tonsillar involvement and two other reports describe nasopharyngeal tumors with the histologic picture of follicular lymphoblastoma in which neither the lymph nodes nor spleen [17 0] were grossly involved Tonsillar involvement with other types of lymphosarcoma is by no means rare

Case 6 S W a male Chippewa Indian 60 years old was admitted to the Wisconsin General Hospital on April 29 1943 complaining chiefly of weakness He had been in good health until six weeks previously when he began to suffer from weakness which became progressive Four weeks before admission he had gone to his local doctor and a week later he noted swelling of the penis and scrotum which progressed Three days before admission he developed swelling of the left leg In response to questioning he revealed that for two years he had noted enlarged nodes in the left inguinal region and similar nodes on the right for four or five months During the past six weeks nodes in the axillary and cervical region and an abdominal mass became apparent There had been a nonproductive cough and for seven weeks intermittent tarry stools The patient had had pleurisy at the age of 21

Physical examination showed a moderately well-nourished man Scattered in the cervical supraclavicular and axillary regions were moderately firm discrete nodes 1 to 2 cm in size and there was a tiny left epitrochlear node There were similar nodes 2 to 3 cm in diameter in the inguinal regions and a large firm mass filled most of the left side of the abdomen Expansion of the left lung was limited and there was dullness at the left base The liver and spleen were not palpable The penis scrotum left lower extremity to the mid thigh and the right foot were moderately edematous

Laboratory studies showed a hemoglobin of 13 3 Gm (80 per cent) erythrocytes 4 250 000 leukocytes 6 700 with neutrophiles 64 per cent lymphocytes 29 per cent monocytes 2 per cent and eosinophiles 5 per cent Blood Wassermann was negative Hanger s cephalin-cholesterol flocculation test was negative Roentgenogram of the chest showed atelectasis at the left apex with displacement of the trachea to the left There was extensive fibrosis and some calcification of the pleura with a small left pleural effusion

* The biopsy section of April 3 1945 has been reviewed and the diagnosis of reticulum cell lymphosarcoma confirmed

The mediastinal shadow was widened and radiating areas of infiltration extended into the field of the lower left lung X ray of the colon following barium enema showed marked narrowing of the sigmoid apparently due to an extrinsic mass Gastrointestinal roentgenograms showed that the esophagus deviated sharply to the left in its upper portion and that the trachea was displaced in consequence of the fibrotic and calcified pleura of the left apex The stomach was displaced upward because of abdominal masses and fluid The duodenal loop was large and rounded probably as a result of a mass of nodes about the head of the pancreas Biopsies were done of an inguinal node of an epitrochlear node and later of an axillary node Large hyperplastic follicles were noted which almost crowded out the pulp lymphocytes Dr W D Stovall interpreted these nodes as malignant giant follicul oma (follicular lymphoblastoma)

The patient after a period of twenty essentially afebrile days in the hospital during which time he received eight treatments of 200-r each of roentgen therapy (H V L cu = 2 4 mm distance 50 cm) to the anterior and posterior mid abdomen was discharged on May 19 He had been seen in the hospital and in the outpatient department at intervals of about three months to December 11 1946 his hospital admissions totaling ten He had continued to have quite general enlargement of the lymph nodes and on his second admission the spleen and liver were enlarged At times superficial nodes were as great as 5 to 6 cm in diameter Roentgen therapy to various sites had been given with each admission On several occasions abdominal paracentesis resulting in the removal of as much as 3 000 cc of creamy foul smelling fluid was done Temporary improvement followed each course of therapy In August 1945 when he was admitted for the seventh time he complained of having suffered shooting pains in the legs for ten days making it impossible to walk For four days previously he had walked dragging his feet He could move his toes slightly Examination at this time showed scattered superficial nodes 1 5 to 3 cm in diameter persistence of pulmonary changes and marked weakness of the legs though he could move them a little in bed The knee jerks were present but Achilles reflexes and abdominal reflexes were absent Babinski and confirmatory signs were present bilaterally There was tenderness over the spine at the level of the sixth and seventh dorsal vertebrae Spinal tap was done and there was evidence of block

Laboratory studies showed a hemoglobin of 10 7 Gm (69 per cent) erythrocytes 3 030 000 lymphocytes 4 240 *with a relative lymphocytosis Spinal fluid showed a negative serology gold sol 0011233* 2100 no cells sugar 63 mg protein 415 mg per 100 cc of blood X ray of the spine showed no alteration in the vertebra but roentgenogram of the chest showed distinct increase in the mediastinal mass with metastatic nodules in the right pleura The patient received four roentgenographic treatments of 200-r each to the anterior and posterior mediastinum and was discharged as improved He gradually regained some strength in his legs but in October 1945 was still unable to walk On this admission the hemoglobin was 8 2 Gm (50 per cent) erythrocytes 2 350 000 The chest x ray showed a marked decrease in the width of the mediastinal mass but an increase in the size and number of the metastatic nodules on the field of the right lung Further roentgenotherapy was administered to the mediastinum

The patient was next admitted on August 1 1946 complaining of pain and weakness about the right knee and in the right leg which was relieved by rest He was thin and pallid There were scattered enlarged lymph nodes 2 to 4 cm in diameter There was a 2 x 2 cm tender nodule on the medial side of the right femur just about the knee Roentgenogram of the right femur at the junction of the middle and lower thirds showed an osteolytic lesion involving the shaft of the femur for a distance of 7 to 10 cm A large central rarefaction extended almost through the cortex laterally and there were a number of smaller rarefactions which were intercommunicating Pathologic fracture was thought to be imminent The patient was placed in a hip spica cast and seven treatments of 150-r each in air (H V L cu = 1.05 mm to 2 40 mm distance 50 cm) were administered to the femur through a window in the cast As the right testis was enlarged irregular and hard and was believed to be involved by tumor a small amount of therapy was directed to it also

The patient next entered the hospital on November 26 1947 The pain in the right lower extremity persisted but was less severe and less constant The patient was thin and pallid A few superficial nodes were palpable There was tenderness of the left fifth rib The cast was still in place on the right lower extremity Hemoglobin at this time was 11 3 Gm (70 per cent) erythrocytes 4 320 000 leukocyte count 2 650 with 56 per cent neutrophiles and 8 per cent eosinophiles The basal metabolic rate was plus 13 and plus 15 Serum proteins were albumin 4 9 Gm and globulin 2 1 Gm per 100 cc Roentgenogram of the chest showed no evidence of recurrence of the mediastinal mass X ray of the right femur showed increased destruction of the cortex in the distal portion of the femur Biopsy of a right epitrochlear node

which was made on December 3 showed the outlines of giant follicles (three and a half years after the inguinal biopsy) which were interpreted by Dr Walter Jaeschke as follicular lymphoblastoma After five treatments of 200-r each in air (H V L cu = 2 4 mm distance 50 cm) to the femur through a window in the cast the patient was discharged The last admission was April 17 1947 The patient at this time was emaciated Biopsy of the bone lesion showed several small cellular foci composed of closely packed



Fig 3 Lateral and A P Views of the Femur Case No 6

small cells apparently lymphocytes There were no follicles The interpretation of Dr Walter Jaeschke was necrotic lymphoid tissue

The patient died April 25 1947 The autopsy was done by Dr John W Harman of the Department of Pathology and he reported that the autopsy demonstrated a large firm diffuse greyish white retroperitoneal mass which included both adrenal glands infiltrated the pancreas was attached to the under surface of the liver and surrounded and constricted the pelvi ureteric junction of the left kidney There were several discrete nodules of similar tissue in the liver (see fig 4) The only enlarged lymph nodes seen were the left epitrochlear and right external iliac The spleen weighed 250 Gm Microscopically the

retroperitoneal mass liver nodules and enlarg d lymph nodes ha l a predominantly follicular structure The large follicles v ere widely separated by diffu e areas of small lymphocytes and were composed of similar cells them elves By reticular stain the f llicular structure was accentuated ea h follicle was surrounded by a zore of compres ed reticulin fibers In all sections the cell type was almost exclusively



Fig 4 Section o the Liver Showing Nodules From the Autopsy Case No 6

small lymphocytic only ra e clasmatocyte we e seen The splet i structure was normal the follicles were few small an l idely separated by the pulp Liagno is malignant follicular lymphoma

Comment A male Indian with follicular lymphoblastoma which may have begun in 1941 and was diagnosed in 1943 evidencing widespread disease with probable involvement of the spinal cord and definite involvement of bone with resultant destruction The architecture of the lymph nodes had changed but some char

acteristics of follicular lymphoblastoma persisted in the biopsy done in December 1946, although the patient s condition was poor and the disease far advanced The development of a bone disease such as this strongly suggests that we are dealing with a malignant tumor such as a lymphosarcoma Finally at autopsy four years after onset of the illness there was still the characteristic pathologic change of the follicular lymphoblastoma

Discussion

The six cases herein described represent varying symptomatic states dependent upon varying sites of the lesions and emphasize the protean manifestation of follicular lymphoblastoma Two cases No 5 and No 6 present previously rarely reported or unrecorded lesions

Analysis of these cases tends to confirm observations made by Baehr Klemperer and Rosenthal[11] and by Baggenstoss and Heck [10] The disease tends to but does not invariably pursue a relatively slow course The onset is ordinarily insidious Pleural effusion was prominent occurring in 3 of the 6 cases Ascites was demonstrated in case No 6 Hypochromic anemia was almost invariably present when the patient was seen late rarely so if seen early The leukocyte counts showed nothing characteristic early or late Cachexia was unusual until late in the disease This is quite characteristic of other types of lymphosarcoma however whereas anemia appears relatively early in Hodgkin s disease The course in those of our cases followed for long periods certainly suggests that one is dealing with a neoplastic process malignant in character Bone destruction prominent in one case No 6 and involvement present in another No 2 further suggests the malignant character of follicular lymphoblastoma In a word our observations although not extensive tend to confirm the conclusion of Baehr Klemperer and Rosenthal[11] and Baggenstoss and Heck[10] that this is a form of lymphosarcoma and that although readily amenable to roentgen therapy it is not ordinarily curable by this type of therapy as has been suggested [31]

This series does not enable one to form an opinion as to the duration of the disease but general opinion holds that the course is slower than with the other forms of lymphosarcoma Of our cases No 6 has been ill for four years and may have had lymph node involvement for six years Case No 1 whom we observed from early in her course to almost the end survived about eighteen months Baggenstoss and Heck[10] have discussed well the pathologic differentiation of follicular lymphoblastoma and lymph node hyperplasia of inflammation and our observations add nothing in this regard Evans[32] has listed the pathologic differences of lymphadenitis of secondary syphilis and follicular lymphoblastoma

Conclusions

Six cases of follicular lymphoblastoma are here reported They demonstrate a variety of pathologic lesions with a characteristic histologic picture with variable symptoms and signs Tonsillar involvement was unique as the sole demonstrable lesion in one Bone involvement occurred in two

Study of these 6 cases leads us to believe that we are dealing with a clinical and pathologic entity and a malignant tumor one which is usually highly sensitive to roentgen therapy but ordinarily recurrent and progressive

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PART VI

BIOLOGIC AND CYTOLOGIC REACTIONS OF BLOOD AND BLOOD-FORMING TISSUES

56

THE SPAN OF LIFE OF THE RED BLOOD CELL A RÉSUMÉ

By WINIFRED ASHBY PH D

THE QUESTION of the life span of the red blood cell has been an open one for the past hundred years Beginning with naive attempts to determine the duration of the corpuscle in the circulation by transfusions of foreign nucleated cells into the mammalian blood stream [1] the quest continues as of November 1946 with the most modern biologic tool the isotope and with full benefit of higher mathematics in the analysis of data collected A solution at last seems in sight

The fact that the mammalian red blood corpuscle is an incomplete cell which has lost its nucleus together with the fact that it is of necessity subjected to the stress of contortion as it passes through the capillaries has made its ephemeral existence an acceptable hypothesis To anyone who has had the privilege of seeing the beautiful moving picture made by Professor August Krogh showing the opening and contracting of the capillaries and of the red blood cells being crowded through them it is amazing that the frail erythrocyte should survive for any great length of time

Two schools of thought have arisen concerning this subject One is influenced by the seeming fragility of the corpuscle irreconcilable with any long buffeting in the circulation Its members still accept the earlier work upon the rate of bile pigment excretion which argues for a short life of the cell [2] The chief protagonist of this school is Dr Raphael Isaacs His expressed opinion as of 1938 was as follows As the erythrocytes in mammals are non nucleated it is evidently impossible for repair and metabolic nutrition to be a part of their normal physiology Their life duration must be limited and is probably shorter than most of the fixed tissue cells but the exact or even approximate length of service is not known [3] This opinion is still acceptable to many authorities in the field of blood physiology and has appeared in some of the postwar textbooks in which the physiology of blood destruction is discussed For instance in An Integrated Practice of Medicine [4] (1946) edited by Harold Thomas Hyman we find the following statement on page 1038 The life of the normal erythrocyte is very limited and probably does not exceed a span of more than six weeks

The other school bases its judgment upon certain technics which tag the corpuscle either directly or indirectly and enable the observer to follow it until it is eliminated The members of this school accept notwithstanding its seeming improbability a life span of about 120 days for the red cell in the normal body

THE METABOLISM OF THE MAMMALIAN RED BLOOD CELL

Since this supposed frailty of the non nucleated erythrocyte has played so great a part in our thinking concerning the span of life of the red blood cell it would

From St Elizabeths Hospital Washington D C

seem well to examine the evidence which concerns the position from the point of view of metabolism which the red cell holds in the society of cells which compose the mammalian organism

In the first place, although the nucleus is necessary for cell division and reproduction, it is not necessary for the continued life of the cytoplasm This has been beautifully demonstrated by Dr Chambers especially in his work upon the enucleated ameba, which survives for a long time forming contractile and gastric vacuoles The nucleus is not as one was inclined to think in one s high school days the brain which directs the metabolic activities of the cell The cytoplasm also has its enzymes by which its metabolic activities are accomplished One thinks of the various components of the cell as being produced in situ from previous material of the same kind For instance botanists have long regarded the plasted that component of the cytoplasm in which starch is produced when the leaf is illuminated as being capable of reproducing itself and of passing on as such when cell division occurs [5] In the recent conference on the chemistry and physiology of growth held at Princeton the question was proposed as to the passivity of the cytoplasm and attention was called to self perpetuating cytoplasmic entities in lower forms which could be passed from the cytoplasm of one cell to another, dependent to some extent upon the genes of the host [6] In line with this are recent disclosures by way of the isotope To quote Addison Gulick Studies with radioactive nitrogen have demonstrated that proteins in the living cell are constantly undergoing exchanges of their amino acid residues Thus even after it is completely synthesized the protein molecule does not maintain its absolute identity of material but merely a steady state with like configuration of like materials [7] The same author mentions the fact that the cytoplasm has its own nucleoprotein which is different from the nucleoprotein of the nucleus but is of the same type as that found in viruses or at least in the tobacco virus And the tobacco virus is not only capable of maintaining itself but given the cooperation of a suitable cell, of very extensively reproducing itself Red cells are reported to contain nucleoproteins They also contain catalysts such as phosphatase carbonic anhydrase coenzyme choline esterase catalase urease lipase Barron[8] quotes some work indicating that red cells can synthesize flavin adenine-dinucleotine from riboflavin in vitro and in vivo It would seem then that the long cherished conception of the histologically minded hematologists that the red cell is a passive hemoglobin containing sac with little other content will have to be modified

We have several indirect points of evidence to the effect that the red cell may be able to repair itself For instance a small amount of glucose the prime source of metabolic energy aids in preventing deterioration of drawn blood Maizels has shown that glucose inhibits the breakdown of organic phosphates in the stored cells [9] Maizels and Paterson showed that stored cells that had taken up sodium at the expense of their normal potassium although they still possessed this excess after half an hour in the blood stream lost it in twenty four hours against the steep concentration gradient produced by the content of the patient s serum They consider that the cells have been reconditioned in a more complex manner than would be accounted for by a simple physical process [10] There is also the loss of the

specific polysaccharides constituting the a and b antigens which takes place when cells are stored under the stabilizing influence of a lowered temperature and removed from the strain of the circulation Whereas under the stress of the circulation the high degree of agglutinability is maintained This would suggest that in vivo the corpuscles have capacity to replace the loss of these specific polysaccharides

The very low metabolism found by Warburg at least in the corpuscles of man is rather against the idea of the synthetic repair of the red cell in the blood stream since energy is required for the building of protein and of the polysaccharides [11] Ramsey and Warren find the respiratory rate of red cells to be comparable to the rate of other resting tissues (30-70 mm^3/Gm/hr) [1] G P Wright finds that normal orthochromatic erythrocytes have a negligible respiration if any but that oxidation increases with increases in reticulocytes and is in proportion to their number present [13] Similar findings had previously been reported by Barer Needle and Baldridge [14] The argument in favor of any metabolic repair in vivo would be in a bad way if it were not for the work of Harrop and Barron who showed that non nucleated erythrocytes respire to an extent comparable to the respiration of other tissues in the presence of a hydrogen acceptor They used methylene blue [15] Following this Michaelis and Salmon found in aqueous extracts of the body tissues notably the liver something which acted in a manner similar to methylene blue and raised the respiratory rate of the red blood cells to the level of tissue cells [16] It would seem almost certain, then that in vivo the metabolism of the red cell is such that the cell would have the energy necessary to repair its own deteriorations

The Study of Transfusions and Hemorrhage

The first attempts to determine the life of the erythrocyte that from the point of view of our present knowledge must be considered seriously were the studies of transfusions or of hemorrhage using changes in the total blood count as the indicator Hunter [17] in a report before the Royal Society of Edinburgh in 1885 of his own work in this line and of that which preceded his gives the results obtained by Ward Miller Quinck and von Ott Ward Miller transfused dogs and produced a plethora After two or three days he judged that the number of corpuscles corresponded to the number transfused plus the original count After a few weeks the count had returned to its first level Hunter in order to avoid the abnormal condition of plethora injected blood intraperitoneally von Ott removed large amounts of blood injected defibrinated blood and observed the time taken for the total count to drop to a minimum The period of observable change indicated by this work was between fourteen and twenty six days

Various modifications of the above type of experimentation have continued to date in 1934 Escobar and Baldwin introduced a new facet in that they produced the plethora by exposure to low oxygen pressure [18] They also measured plasma volume They did not count reticulocytes which Rous and Robinson[19, 20] found reduced to zero in their studies of increase in fragmented corpuscles produced with plethora They did not allow the cell count to increase under oxygen need beyond a certain arbitrary amount because they said If the cell count is too great the

compensatory erythro destroying mechanism begins to act and the supernumerary cells under observation may not live out their allotted normal life span The results of these workers are in good agreement with other work in which this general method has been used In rats the count returned to normal in from twelve to eighteen days, in the dog from sixteen to twenty three days in man from eighteen to thirty days

In all of this work which deals with the whole blood there are three underlying factors and probably a fourth, two of which are quantitatively indeterminate and therefore vitiate any quantitative results The quantitatively indeterminate factors are blood destruction and blood production In the reticulocyte count we have a qualitative index of blood production but there is no way in which the number of reticulocytes can be interpreted in terms of the number of red cells that are being produced The length of time that an increased count remains above normal could be taken as an index of the length of time that the cells producing this increased count remained in the circulation only if we were certain that no change had taken place in the relative rate of production and destruction which maintained the original cell count This we cannot assume In fact we have evidence in the work of Rous and Robertson [1] that plethora causes a reduced production as shown by absence of reticulocytes and by a reduction in the count of cells in the bone marrow and also an increased destruction as shown by the increase of schizocytes in the spleen Both increased destruction and decreased production would cause the count to return to normal without the necessary destruction of the cells introduced to cause the excess counts especially if the excess cells were the youngest cells in the community as would be the case in the experiment of Escobar and Baldwin The duration of the plethoric count would always be shorter than the life of the cells which produced it

In 1930 Eaton and Damren made an interesting contribution to the problem They subjected rabbits and dogs to fairly severe hemorrhage and studied the appearance of reticulocytes The curve of their appearance showed marked crests which gradually subsided through five or six cycles The interval between crests in the dogs curves was approximately sixteen days and that of the rabbits eight and one half days They infer that the blood which is produced in the first crest is destroyed and its destruction is the stimulus for the second crest and so forth

These data would be strong evidence in favor of the shorter term of life of the red cell if there were not some indication that activities of the organism do not proceed at a constant rate but are subject to more or less regular fluctuations From this point of view these data could be explained as magnifications of these tendencies to periodic increases in growth activity caused by the stimulus of increased need This tendency to rhythmic fluctuations in the anabolic and catabolic activities of the organism which appear to become more evident under stress needs to be recognized clearly in attempting to evaluate the evidence concerning the length of life of the red cell

Ashby in 1921 reported that Group O corpuscles transfused into a recipient with agglutinable corpuscles disappeared in steplike intervals that might be from two to four weeks in span [3] Wearn Warren and Ames using the same technic

failed to observe these abrupt changes but they admitted that their observations may not have been sufficiently closely spaced to catch them [21] Of course there are many rhythmic phenomena which have been studied in relation to the menstrual cycle but there is also evidence that such rhythms occur independently They may be seen in weight curves in persons on a reducing diet The cross striations on the fingernails have been attributed to such fluctuations in growth Upon measuring the growth of a series of fingernails through several weeks I have found such fluctuations at intervals of from twelve to fourteen days [3] Schultz in a study of fetal growth in man states that the rates of growth of different parts of the body are frequently found to alternate during fetal life which indicates a certain fluctuation in the rate of growth (p 391) [16] One of the most careful studies of these fluctuations was made by Broun McMaster and Rous [7 24] They used this phenomenon in their study of the relation between blood destruction and the out put of bile pigment They describe it as slow wavelike changes in hemoglobin content and bile pigment output with often as much as a fortnight elapsing between crest and crest If we are willing to accept the longer life of the red blood cell then the findings of Eaton and Damren also become evidence for a periodicity in the activity of the hemopoietic system

Bile Pigment Metabolism and the Span of Life of the Red Blood Cell

Since bile pigment is derived from hemoglobin it is inevitable that measure ments of bile pigment excretion will have played a large role in our estimations in the life of the red cell

The earlier conceptions of bile pigment as derived exclusively from the break down of hemoglobin and as being excreted in its entirety give the red cell a short life when calculated on the basis of the known quantitative relationship between hemoglobin and bile pigment Wilber s hypothesis of a conservation of the pyrrole complex for the formation of fresh hemoglobin by reabsorption from the intestine would still further reduce the figure for life span of the red cell It was not until Whipple and Hooper introduced the conception that bile pigment might be derived from other sources than hemoglobin that evidence of a longer life of the red cell became tenable

Following the earlier work in which calculations were based upon a direct relationship between bile pigment excreted and hemoglobin destroyed Eppinger in 1913 [8] Wilber and Addis [9] in 1914 and Addis[1] in 1915 emphasized the probability that bile pigment was reabsorbed from the intestine and used for the formation of hemoglobin as the needs of the organism demanded This theory led to a great deal of experimental work some of which appeared to support it and some to disprove it

In 1917 Hooper and Whipple[10] published the results of some work on dogs which they considered indicated that bile pigment was not absorbed from the intestine They studied dogs with biliary fistulas to determine the normal rate of bile pig ment excretion after which they injected from 100 to 300 cc of fresh bile by stomach tube They determined the rate of excretion of bile pigment for six hours and in some cases for fifteen hours afterwards They found no significant increase

In 1923 Broun, McMaster and Rous[31] repeated this work with an improved technic for collecting the bile which enabled them continuously to obtain the whole twenty four hour specimen They worked with dogs and part of their experiments consisted in the injection of sheep bile which contained cholehematin a substance not found in dog bile They found this in the dog s biliary secretion and considered this partial evidence that the sheep bile had been taken up from the intestine although they admitted that cholehematin was an extrinsic substance obtained from the green food of the herbivorous animal They also fed dog bile but in this work their results were not consistent In some instances they considered that their figures indicated an increase in the output from the biliary collection upon feeding bile in others no increase was shown They considered that on the whole their results indicated an enterohepatic circulation of bile pigment

In 1926 Bollman Sheard and Mann[3] brought a fresh technic to the solution of the question They compared the bilirubin contents of the venous and arterial blood supply of various organs including the intestine They used fasting animals animals on a mixed diet of meat bread milk and syrup animals given a feeding of cream and egg yolk and animals given fresh dog bile in amounts of from 100 to 200 cc injected into the duodenum the jejunum and the ileum Comparisons were made of the bilirubin contents of the blood from the mesenteric veins and arterial blood at periods of from thirty minutes to three hours after administration of the bile and at suitable periods in the fed animals They had previously found that blood samples withdrawn simulatenously from different arteries of the body contain identical amounts of bilirubin The bilirubin content of blood from venous sources taken at the same time is greater than or identical with that of arterial blood depending upon the areas drained by the veins The findings indicated that bilirubin was added to the blood in the spleen the bone marrow and the liver With the same technic they were unable to detect any increase in the blood return ing from the kidney skeletal muscles or the intestine They conclude that since their method was sufficiently sensitive to detect the addition of bilirubin to the blood as it passes through any of the sites of bilirubin formation no intestinal absorption occurs under the conditions of their experiment

Probably the latest contribution to this problem as to whether the bile pigment is absorbed and reutilized comes from some work by Shemin and Rittenburg[33] using the isotope N^{15} The isotope was incorporated into glycine which had in previous work been shown to be the nitrogenous precursor of the protoporphyrin of hemoglobin When the isotope containing glycine was fed to man the N^{15} con tent of the hemoglobin rose sharply stayed on a level and then fell abruptly to base The flatness of the curve indicates that there was no reutilization of the labeled nitrogen for hemoglobin formation

Having apparently disposed of the question of bile pigment reutilization we come to the even more important one in our assay of the life of the red cell that of whether or not the bile pigment complex has other sources than hemoglobin

In 1916 Whipple and Hooper[34] reported that bile pigment excretion was in creased with carbohydrate feeding By 1922 the studies on the effect of food had been greatly extended by the workers constituting this group It was shown that

red meat cooked liver hemoglobin and butter fat had a positive effect upon the production of hemoglobin or bile pigment Next came spinach full diets of common grain foods and milk Chlorophyl clams onions lard and codliver oil were inert At this time Whipple[35] formulated the conception of a bile pigment complex which would be an intermediary stage in the development of bile pigment or hemoglobin and would be utilized according to the needs of the organism He considers that bile pigment output is a result of functional activity of the liver and not solely the result of the passive elimination of defunct hemoglobin At this time he characterized pernicious anemia as a disease which showed abnormal tendency to pigment production rather than to cell destruction Preceding this Ashby due to a failure to find any increase in destruction of transfused red cells in that disease had suggested that a retardation in the maturation of the red cells in the bone marrow might account for the accumulations of iron because of non utilization and that the physiologic stimulus of the anemia might account for the extension of the active bone marrow and the increase in bile pigment production [36]

In 1923 Rous Brown and McMaster[37] published a series of papers on studies on total bile using their technic of continuous bile pigment collection One of these papers was devoted to the question of the relation of carbohydrates to the output of bile pigment They considered that their results did not substantiate those of Hooper and Whipple They found an increase in bile pigment production upon carbohydrate feeding followed by a compensatory decrease They explained this result on the basis of a temporary hastening of the evacuation of the bile pigment from the liver due to deposits of glycogen In a paper on the relation between blood destruction and the output of bile pigment they described work in which dogs had been intubated with the total removal of bile for periods of three months [38] A secondary anemia developed which showed intercurrent fluctuation accompanied by similar fluctuations in the bile pigment output The relations between these fluctuations were studied In addition some of the dogs were subjected to treadmill exercise and to transfusion of citrated blood The authors find that the output of bile pigment is not fully reflected in the fall in hemoglobin which they interpret as blood destruction They attribute this to a process of pigment conservation which varies in proportion to the body need They add that the destruction finds expression in terms of bile pigment and practically at once and the data support the conception that bilirubin has no other source besides the hemoglobin of destroyed blood

In the evaluation of all such data as the above we have certain inherent difficulties Bile pigment can be measured but blood destruction cannot The blood count is the resultant of two unknowns the rate of blood production and the rate of blood destruction With a decrease in blood production the total red cell count would go down without any change in the rate of blood destruction If we interpret these increases in bile pigment production which Rous and his co workers have found associated with the falls in circulating hemoglobin as part of a stimulus to production of the pyrrole complex in answer to the need they become equally potent arguments for the extra hemoglobin source of bile pigment

It would appear that Hawkins and Whipple[39] have finally settled the question

by pinning one of the unknown factors blood production They did this by removing within a short space of time enormous amounts of blood from dogs Upon regeneration of the red cells they had a population of corpuscles that were of practically the same age They varied the procedure by destroying the blood with acetyl phenylhydrazine to avoid removal of material that might be useful in rebuilding corpuscles The bile pigment output changed from 125 mg per day to 58 mg and remained at this low level because the population of the blood stream was preponderantly young cells that were not being destroyed After approximately four months the bile pigment rose to a high level This was interpreted as being the result of the eventual death of the cells that had been formed after the massive hemorrhages The life span was estimated at 133 days

The Use of Differential Agglutination

After the establishment of the human blood groups by Landsteiner followed by Jansky and Mo s much clinical experience was accumulated with reference to the blood transfusion By 1918 the question was in debate by the medical profession as to whether or not transfused blood existed in the circulation for even the twenty two days suggested by the work summarized by Hunter or, whether it was removed within a few days and any favorable clinical results were due to the supply of raw material from which new cells could be formed more readily

In 1911 an article by Charles Todd and R G White[40] had appeared in the proceedings of the Royal Society On the fate of the red blood corpuscles when injected into an animal of the same species The authors used a highly polyvalent isohemolytic cattle serum produced by injection of cattle with the blood of other individuals By this technic they had previously reported a high degree of individuality in cattle similar to that found by Ehrlich in goats This serum was exhausted for the antihemolysins of the corpuscles to be studied It was used to separate transfused blood from the blood of the recipient The transfused blood was found to disappear in the course of a few days The authors found that the injected corpuscles are treated by their host as foreign and in fact act as antigens and give rise to the formation of corresponding antibodies in accordance with the ordinary laws of immunity

In 1918 Ashby[41] at the Mayo Clinic taking advantage of the difference in agglutinability of the recipient and donor cells in instances of transfusion with the *universal donor published the result of the study of 3 cases in which it was* claimed that the transfused blood stayed in the circulation for considerable lengths of time time enough to produce beneficial results due to functioning cells Because of the rapid turnover of patients at the Mayo Clinic it was difficult to follow the full span of life of the transfused blood and nothing was postulated as to this point Of the 2 cases reported which remained under observation for forty days in one that of a woman who had had a total hysterectomy the transfused blood had come to base while in the other a man who was transfused for simple hemorrhage there was little sign of disappearance of the transfused blood at the end of this time Included in this first report were studies checking the quantitative validity of the technic with in vitro mixtures of bloods and certain points necessary for

satisfactory results were stressed There were also included 10 cases studied for only a short time in which the data indicated a common factor relating the amount of blood transfused the body weight of the patient and the number of cells found in the blood after transfusion attributable to the transfused blood as determined by differential agglutination Such data were subsequently used in the study of blood volume

Three papers by the same author followed this initial report [4, 22, 36] They gave data on approximately 40 cases Many of these patients were suffering from fatal illnesses Although the longer life of the corpuscle was amply demonstrated in a few patients who were in comparatively normal health attention was called to the great irregularity in the time taken for elimination of the transfused cells in a group of patients in which it had been possible to follow the count to its extinction These survival periods ranged from 30 to 110 days It was argued that transfused bloods having been taken from normal donors had equal capacities to survive and that the differences in survival periods were due to an activity of the organism receiving the blood This argument was offered in conjunction with the data in connection with certain cases in which periodic steplike decreases in the count were seen The capacity for the long survival of the red cell in the blood stream, however is seen In 11 cases that were under observation for periods varying from 22 to 51 days during which no appreciable drop in the count of transfused blood took place from the case of a man who was in good health who had been trans fused for a simple hemorrhage and whose count was followed for 110 days when there was still evidence of some of the transfused blood from cases of pernicious anemia in which there was evidence of survival of the last of the transfused cor puscles which were presumably the youngest at the time of transfusion 95 and 100 days after the transfusion had been given

In 1922 Wearn Warren and Ames [4] using Ashby s technic presented prolonged studies on 8 patients from the Medical Service of Peter Bent Brigham Hospital Four were cases of primary anemia and four of secondary anemia due to nephritis They report that the last of the transfused red blood cells disappeared from the circulation in from 59 to 113 days No difference was noted in a series of observa tions in the duration of the stay of the transfused red blood corpuscles in the circulation between patients with primary and secondary anemia due to nephritis and that in a single observation red blood corpuscles from a patient with perni cious anemia transfused into another patient with pernicious anemia behaved as did corpuscles from normal donors

By 1926 several criticisms had been leveled against this apparently simple technic of Ashby notably by Wildegans[43] and Gorl [44] The criticisms were due to an inability to get quantitative separation of in vitro mixtures of agglutinable and nonagglutinable blood as had been reported by Ashby in the original description of the technic These failures to repeat the results of Ashby were probably due to the use of serum of insufficient agglutinating strength or to the use of hemolytic serum both of which were warned against in Ashby s original description of the technic A possible cause also would be insufficient agitation during the initial period of agglutination The technic was not acquired by these workers

The most serious criticism of the findings of Ashby and of Wearn Warren and Ames, indicating a prolonged life for the red cell in the blood stream came from Isaacs, in 1924 [45] He reported The use of agglutination in recognizing the cells of a donor in a mixture of two bloods in a transfused patient is of little value after the number of young cells reaches the number of unagglutinable cells usually in from two to four days This statement was based on work using a radical modification in technic, with no report of in vitro work to see whether or not the technic gave quantitative separation of an agglutinable from a nonagglutinable blood The greater part of the work was done upon the blood of dogs in which animal there are not known to be strong isoagglutinins and for which the author gave no evidence that they exist Work upon 2 human cases was reported the results of which were entirely at variance with the results already reported with the Ashby technic on some 50 cases by Ashby and by Wearn Warren and Ames

Ashby immediately criticized this paper of Isaacs,[46] in the first place because the immature cells which Isaacs had claimed composed the unagglutinated cells were not found among the nonagglutinated cells by the Ashby technic The same finding was reported in 1940 by Maizels and Paterson who say If the unagglutinable cells are immature cells they should show a high retic count higher than that of the whole blood but the reverse is the case In the second place the prolonged rise in the count of unagglutinable cells after a Group O transfusion and the lack of a rise after a like group transfusion reported by both Ashby and by Wearn Warren and Ames was not explained by Isaacs It was also pointed out that on the basis of the amount of blood given and the body weight of the two patients the counts of the unagglutinable blood found by Isaacs subsequent to transfusion would indicate an impossible blood volume relationship being in one case 25 per cent of the patient s body weight and in the other 75 per cent of the body weight It was concluded that Isaacs had not accounted for the transfused blood by his technic

Isaacs results however were considered seriously by the group interested in the longevity of the blood cell and Isaacs himself reiterated them in a paper in Physiologic Reviews in 1937 [48] so that at the beginning of World War II when several English groups were studying the effect of storage upon survival of transfused blood for use at the front Maizels and Paterson[47] undertook to check on the validity of Isaacs claims With reference to Isaacs criticisms of the Ashby technic they state It may be said at once that these criticisms are theoretical and unsupported by numerical data however since the cell agglutination technique is the only one which permits of a direct measure of cell survival the objections must be considered in detail The arguments were approximately those used above except that in addition the M and N factors were used to separately agglutinate the donor and recipient cells

By 1928 however Landsteiner Levine and Janes[44] had offered irrefutable disproof of Isaacs contention for the short life of the cells by making use of their anti M and N sera and agglutinating the transfused cells instead of the recipient s cells They found clumps in the recipient s blood treated with their antiserum for seven weeks after transfusion when they ceased to examine it Later Wiener [50] using

M antiserum with an M donor reported that the life of the transfused cell probably averages between 80 and 120 days

The very considerable amount of work which was done at the beginning of the war checking the various methods for preserving blood and the time allowable for storage made use of the cell agglutination technic This work was carried out by the English Groups,[47 51 5] by Wiener and Schafer[53] in this country and by the Russians in checking their cadaver blood This work all indicated the long survival of the transfused corpuscle

DETERMINATION OF THE LIFE SPAN OF THE RED CELL BY USE OF THE ISOTOPE

The problems involved in tagging the red cell by use of the isotope and determining its longevity are First to introduce the isotope into the corpuscle in such a chemical combination that it remains in the corpuscle during its life second to choose an isotope of some element that will not be reutilized after the corpuscle has disintegrated and last to choose an isotope that can be studied for a sufficient length of time

The interesting phenomenon which the use of isotopes has emphasized is that although morphologically the cell and its constituents are intact the reversibility within the status of their equilibrium of certain cellular reactions, results in continuous degradation and resynthesis An isotope introduced into a constituent of the cell which bore such a relationship to the metabolism of the cell would not indicate by its presence the life of the cell but would merely be an index of the time relationship of the reaction in which the compound was involved

On the other hand if the isotope is some element which will be reutilized in the formation of new cells its presence will extend beyond the lifetime of the cells into which it was first introduced It has long been considered probable that iron split off from hemoglobin upon the formation of protoporphyrin is quite labile and is readily reutilized In 1941, Cruz Hahn and Bale [54] working with radioactive iron reduced the iron reserves of dogs by repeated hemorrhage and fed radioactive iron This appeared in the blood with the rise of hemoglobin and stayed on a level for seventy five days when the dogs were treated with acetylphenylhydrazine The resultant drop in hemoglobin was paralleled by a drop in the radioactivity of the blood which rose to its original height with the regeneration of hemoglobin, The isotope level had not decreased 180 days later The authors conclude that iron liberated from red cells is utilized readily and nearly quantitatively for the regeneration of hemoglobin in new red cells

In 1946 Shemin and Rittenberg introduced the isotope N^{15} into several compounds which were of interest in their possible relationship to the synthesis of protoporphyrin of hemoglobin [55] Glycine proline and glutamic acid were chosen because there was a probability that they would be used in the formation of the protoporphyrin leucine was chosen as a representative α amino acid whose intact carbon chain was unlikely to be used for pyrrole synthesis and ammonia was chosen to test the nonspecific utilization of nitrogen liberated by deamination of amino acids These substances were fed to rats whose hemin was subsequently tested *for the isotope It was shown that glycine is a nitrogenous precursor of the*

protoporphyrin of the hemoglobin of rats The authors were of the opinion that the much smaller amount of N^{15} found in the hemin upon feeding of isotope proline, leucine glutamic acid and ammonia is due to N^{15} enrichment by the nitrogen of these compounds, of the body nitrogen from which the precursors of heme is synthesized rather than a direct utilization of these compounds

Having established this fundamental point these workers proceeded to feed N^{15} containing glycine to a man and to study the shape of the curve indicating the appearance in the circulation of this isotope and its disappearance from the circulation The values rose rapidly to a high level remained practically constant for many weeks and then fell quite sharply to a very low level This finding indicates that the heme is neither involved in the dynamic metabolic state nor reutilized for hemoglobin formation On these grounds the curve of N^{15} concentration of the heme versus time can form a basis for the average life span of the human red blood cell This was found to be about 127 days [33]

Conclusion

We come then after following the evidence derived from three separate approaches to the conclusion that the life span of the red cell approximates the dimension of 110 to 130 days under favorable conditions

This does not mean however that for practical purposes a transfusion lasts for that length of time The blood transfused consists of cells of all ages up to the full span of four months and half of them are already half of this age when they are transfused Neither does it mean that the span of life of the red cell is of this dimension under all circumstances Hawkins and Whipple reported the figure of 133 days from work on normal dogs The study of Shemin and Rittenberg using the isotope N^{15} in which they found the life span to be 127 days was made on a normal man The subjects of transfusion however are usually far from normal therefore the data derived from the cell agglutination technic has to be considered in relation to the condition of the patient transfused Ashby (1921) Wearn Warren and Ames (1922) Wiener (1934) Mollison and Young (1940) and Callender Powell and Witts (1945) [56] are agreed upon 120 days as an approach to an optimum length of life for the transfused red cell as indicated by the method of differential agglutination Ashby (1921) and Wearn Warren and Ames (1922) are agreed that in pernicious anemia the tenure of life of the red cell is of a similar dimension But there are other conditions in which the life span of the transfused blood and probably also of the patient s own blood is of much shorter duration

I have on this occasion reviewed my own studies of transfused Group O blood into recipients with blood of an agglutinable type of which I have over 80 cases with few unfortunatley studied to the extinction of the transfusion I have endeavored to draw some conclusions from these data by extending the slope of the curve of elimination to a base line This is admittedly not accurate in the individual case but appears to give interesting average results In 8 cases uncomplicated by malignancy in which postoperative transfusions were given the average time taken for unagglutinable cells to come to base was 124 days In 16 cases of pernicious anemia it was 110 days In addition there were in these groups cases which

showed no drop in the count while under observation and therefore no estimation could be made On the other hand 10 cases with malignancy averaged 52 days Seven cases of jaundice averaged 46 days In a case of aplastic anemia with smallpox studied for 26 days the curve of the count of transfused corpuscles would have come to base in 41 days while a case of splenic anemia in an infant by this method showed elimination of a first transfusion in 44 days and of a second in 55 days In a total of 24 cases in which hyperthyroidism and severe chronic infection are included the average of the apparent length of life of the transfused blood was 52 days But in 5 instances in which death was imminent the life of the transfused cell was even shorter

It would seem then that we will have to regard the erythrocyte not as an entity but as an integral part of the organism Castle and Minot[37] in their article on the anemias in Oxford Medicine introduce Boycott s conception of the erythron which is understood to be the circulating blood and the organ from which it arises I believe however that before the picture is complete we will have to consider a broader angle and include the effect of the endocrine system As we pointed out in the beginning of this review the red cell both the compatible transfused cell and that produced in the body probably is capable of undergoing repair and it is by virtue of its capacity to repair itself that it survives the buffeting of the circulation In a body in which the anabolic processes as compared with the catabolic processes are on the down grade as in the terminal stages of disease or in the presence of malignancy one would expect the red cell to suffer with other body tissues and its life in the blood stream to be shortened

The new knowledge on the interplay between the cortical and anterior pituitary hormones and their control of the up-build and destruction of protein and their relation to insulin in carbohydrate metabolism as illustrated by the work of the Cori s on the enzyme complex hexosekinase may be intimately related to the problem of the life of the red cell This question has already begun to interest biochemists In the 1946 meeting of the Federation of American Societies for Experimental Biology a study was reported by Gonzales and Angerer[58] on the effect of adrenalectomy on the respiration of the erythrocytes of rats They found a 42 per cent decrease in oxygen consumption in a Kreb-Ringer suspension of washed rat red cells five days after adrenalectomy and noted that the decrease was perceptible within forty-eight hours

If we may assume that the life of the red cell in the rat is longer than five days which would seem to be a safe assumption this finding would indicate that the erythrocyte is dependent upon the adrenal for something that maintains its capacity to utilize oxygen and brings it into relationship with the organism in its ability to maintain itself in the circulation

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OSMOMETRIC BEHAVIOR OF NORMAL AND ABNORMAL HUMAN ERYTHROCYTES

By George M Guest, M D

VARIOUS investigators have postulated two main principles which seem govern the swelling and hemolysis of red blood cells suspended in hypot salt solutions namely the cells imbibe water according to the laws of osm and their maximum swelling is limited by an inelastic surface membrane Ac ing to this concept hemolysis occurs when the erythrocytes in hypotonic solu attain a critical volume at which the cells rupture and allow hemoglobin to es Studies of the physico chemical mechanisms involved in the processes of osi swelling and hemolysis of erythrocytes have involved a great deal of contro especially over the question of whether the mammalian red cell behaves as a fect osmometer adapting itself to changes in osmotic pressure of surrou fluids by the transfer of water alone (Ponder [18]) Evidence assembled by Por from many sources indicates thar the erythrocytes of various species sometir behave as perfect osmometers but at other times and under varying cond they behave as decidedly imperfect osmometers The reasons for this varial havior are still obscure

Methods devised for the measurement of the osmotic fragility of red cell had clinical applications especially valuable in the diagnosis of congenital lytic jaundice and in the differential diagnosis of other types of hemolytic d The first development of such a method is generally ascribed to Hamburger in 1883 devised a test that involved the notation of beginning hemoly complete or total hemolysis or the points of minimal and maximal resi of red cells suspended in a series of solutions of diminishing concentrati sodium chloride Jolly[16] states that even earlier in 1880 Chanel descr method for measuring red cell fragility in hypotonic salt solutions and applic clinical studies It is of interest to note that Chanel s was a quantitative t contrast to the essentially qualitative test of Hamburger According to Chanel made suspensions of blood cells in a series of salt solutions of dimin concentrations and after a given time determined the number of cells destro counting the intact cells By this method he demonstrated diminished o resistance of erythrocytes in certain anemias and increased resistance in c types of jaundice Whitby and Hynes[24] independently developed approximate same procedure in 1935 Several refinements of Hamburger s procedure hav suggested to disclose minor differences in cell fragility that characterize c pathologic types of red cells (Wintrobe [5]) but the noteworthy advanc methodology are mainly confined to quantitative measurements of hemc Estimations of the degree of hemolysis at each point in the hemolytic series

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With the Technical Assistance of Mary E Wing M S Miss Wing died April 5 1947

been made by determinations of the hemoglobin liberated from the erythrocytes into the suspension fluids by visual colorimetry[1, 11] and by more accurate photoelectric colorimetry.[1, 3]

Various technics have been employed for demonstrating the swelling or osmometric behavior of various species of red cells in hypotonic fluids: determinations of cell volume have been made by centrifugation employing several types of hematocrit apparatus and by electrical conductivity measurements; estimations of mean cell diameters have been made by diffraction methods and by measurements of projected images of the cells sometimes with photographs (Ponder[1–3]). Another approach to this study is by kinetic methods (Jacobs et al.[13–15]) whereby the rate of hemolysis of red cells is determined, within periods of a few seconds, in hypotonic solutions at the critical tonicities or less that produce complete hemolysis. Such technics have been used rarely if at all in clinical studies of abnormal types of erythrocytes.

Seeking a practical method for studying the osmometric behavior of red cells in clinical investigations, Guest and Wing[3] at first employed standard Van Allen hematocrit tubes in which blood was diluted in the usual series of salt solutions. After centrifugation the volume of packed cells measured in the graduated stems of the pipets afforded an estimate of the swelling of the cells that occurred before hemolysis, and the progress of hemolysis at lower tonicities was judged by the diminishing mass of cells as well as by the color of the fluid in the bulbs of successive pipets. A later elaboration of that procedure[6] combined the quantitative determination of hemolysis with measurements of the volume of cells at each stage of the test. Observations made by this method on the erythrocytes of normal adults, children and newborn infants indicated that the swelling of these normal human red cells in every instance followed closely that expected in perfect osmometers. Moreover, the maximum volumes attained by the normal cells before hemolysis agreed closely with predictions based on estimations of their mean surface area, thus supporting the view that the red cell cannot distend beyond the limit set by its surface area. The present paper reports further data gathered by that method on the erythrocytes of normal subjects and on the erythrocytes of patients suffering several diseases in which abnormal fragility of red cells is commonly found.

Method

Only the main steps of the procedure and the principal calculations employed need be given here; further details may be found in the paper by Guest and Wing. The special Van Allen pipet with elongated bulb devised for this method is calibrated to contain 8.0 cc. when filled to the upper mark, and to contain 0.02 cc. in the graduated portion of the stem marked with 100 divisions. Twelve or 15 pipets are usually used in each test. Heparinized blood is drawn into the stem to the 100 mark and after it the appropriate solution of NaCl is drawn into the pipet almost to fill the bulb, leaving a small air bubble to facilitate mixing the fluid and blood. The usual series of salt solutions is employed, beginning with 0.9 per cent NaCl, with decrements in concentrations 0.1 or 0.05 per cent before the beginning of hemolysis and 0.025 per cent through the expected critical range of hemolysis. The tip of each pipet is then sealed with a rubber-cushioned spring clip and the pipets are left resting in a horizontal position at room temperature an hour, after which they are centrifuged 20 minutes at 1500 to 3000 r.p.m., and the volume of packed cells is read in per cent on the graduated stem. The bulb is then filled to the 8.0 cc. mark with distilled water (making the dilution of blood 1:400); the fluid is transferred by means of a long stemmed

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VARIOUS investigators have postulated two main principles which seem to govern the swelling and hemolysis of red blood cells suspended in hypotonic salt solutions namely the cells imbibe water according to the laws of osmosis and their maximum swelling is limited by an inelastic surface membrane According to this concept hemolysis occurs when the erythrocytes in hypotonic solutions attain a critical volume at which the cells rupture and allow hemoglobin to escape Studies of the physico chemical mechanisms involved in the processes of osmotic swelling and hemolysis of erythrocytes have involved a great deal of controversy *especially over the question of whether the mammalian red cell behaves as a* perfect osmometer adapting itself to changes in osmotic pressure of surrounding fluids by the transfer of water alone (Ponder [18]) Evidence assembled by Ponder[19] from many sources indicates that the erythrocytes of various species sometimes do behave as perfect osmometers but at other times and under varying conditions they behave as decidedly imperfect osmometers The reasons for this variable behavior are still obscure

Methods devised for the measurement of the osmotic fragility of red cells have had clinical applications especially valuable in the diagnosis of congenital hemolytic jaundice and in the differential diagnosis of other types of hemolytic disease The first development of such a method is generally ascribed to Hamburger[10] who *in 1883 devised a test that involved the notation of* beginning hemolysis and complete or total hemolysis or the points of minimal and maximal resistance of red cells suspended in a series of solutions of diminishing concentrations of sodium chloride Jolly[16] states that even earlier in 1880 Chanel[2] described a method for measuring red cell fragility in hypotonic salt solutions and applied it in clinical studies It is of interest to note that Chanel s was a quantitative test in contrast to the essentially qualitative test of Hamburger According to Jolly Chanel made suspensions of blood cells in a series of salt solutions of diminishing concentrations and after a given time determined the number of cells destroyed by counting the intact cells By this method he demonstrated diminished osmotic resistance of erythrocytes in certain anemias and increased resistance in certain types of jaundice Whitby and Hynes [4] independently developed approximately the same procedure in 1935 Several refinements of Hamburger s procedure have been suggested to disclose minor differences in cell fragility that characterize certain pathologic types of red cells (Wintrobe [5]) but the noteworthy advances in methodology are mainly confined to quantitative measurements of hemolysis Estimations of the degree of hemolysis at each point in the hemolytic series have

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the medium to 0 9 per cent NaCl solution (i e with 0 45 per cent NaCl $T = 0\ 5$) 100 = the initial volume of the cells In the last column the R values represent

TABLE 1 —*Osmotic swelling and hemolysis of normal human erythrocytes in hypotonic saline solutions*

Normal Subject	Initial Mean Dimensions				Volume of a sphere with same surface area	Tonicity at which maximal swelling occurred	Hemolysis at this tonicity	Expected osmometric volume at this tonicity*	Maximum Cell Volume		R value
	Volume	Diameter	Thickness	Surface					Expected calculated from surface	Final observed (ometer)	
		μ	μ	μ	μ	% NaCl	%	%	%†	%†	
Adults											
1	92	7 7	1 98	141	157	0 450	2 9	170	171	172	1 00
2	90	7 7	1 93	140	156	0 425	24 4	1 8	173	179	1 00
3	98	7 8	2 05	146	166	0 450	32 0	170	169	171	0 96
4	98	7 8	2 05	146	166	0 4 5	37 9	163	169	166	0 99
5	91	7 7	1 93	140	156	0 425	47 6	178	172	180	1 01
6	87	7 6	1 92	137	151	0 450	11 6	170	172	1 2	1 03
7	86	7 6	1 94	137	151	0 450	16 7	170	176	178	1 03
8	80	7 4	1 86	129	138	0 450	19 3	170	172	17	1 01
9	94	7 8	1 9	144	162	0 450	26 0	170	172	174	1 02
10	88	7 7	1 89	139	154	0 425	22 1	1 8	175	175	0 97
11	89	7 4	2 07	134	146	0 450	35 0	170	164	165	1 01
12	89	7 3	2 13	133	144	0 475	26 3	163	162	166	1 01
13	98	7 8	2 05	146	166	0 475	18 0	163	166	165	1 00
Children											
14	82	6	1 81	134	146	0 425	14 6	1 8	178	174	0 99
15	77	7 4	1 79	128	136	0 425	14 4	178	177	1 2	0 97
16	84	7 5	1 90	133	144	0 425	14 8	178	174	1 3	0 99
17	82	7 5	1 86	132	143	0 425	19 8	178	1 4	173	0 97
18	7	4	1 9	128	136	0 425	18 3	1 8	1 7	174	0 99
Newborn infants											
19	109	7	2 34	150	1 3	0 475	10 9	163	159	153	0 95
20	110	8 1	2 24	57	185	0 450	21 0	170	168	165	0 95
21	1 3	8 0	2 25	15	185	0 450	22 7	170	164	163	0 96
22	1 0	7 9	2 24	154	1 9	0 450	10 8	170	163	162	0 93
23	106	9	2 16	152	176	0 425	42 8	1,8	166	168	0 96
24	104	7 9	2 2	151	174	0 4 5	22 3	178	167	168	0 95

* Volume of a perfect osmometer in per cent of its initial volume assuming the initial water content to be o per cent by volume

† Values in per cent of the initial volume of cells in 0 9 per cent NaCl solution

R value in each case is the average for all points determined in the series through the point of maximum swelling of unhemolyzed cells

the ratio of the observed expected swelling Ponder introduced the factor R in the formula $V = RW (1\ T - 1) + 100$ to reconcile observation with theory after

:t to colorimeter tubes and the hemoglobin content of each fluid is read in a photoelectric color ter [8] The total hemoglobin content of the blood is determined on a separate sample measured into illed water with a drop of ammonium hydroxide added to insure complete hemolysis and absence of idity The hemoglobin content of each supernatant fluid in the series is then expressed in per cent of total hemoglobin indicating the per cent hemolysis found at each tonicity Further calculations are :e to compare the observed cell volumes with the expected osmometric swelling at each tonicity to predict the maximum swelling expected if the cells attain the volume of spheres within the limits heir estimated mean surface area

TABLE 1 —*Hemolysis and changes in volume of normal erythrocytes of blood diluted 1 400 in solutions of sodium chloride Normal child C S*

These data are presented graphically in figure 1

NaCl	Hemolysis	Volume of cells				R value†
		Readings	Uncorrected for hemolysis	Corrected for hemolysis	Expected osmometric vol m	
%	%	%	%‡	%‡	%‡	
0 900	0	38 2	100		100	
0 800	0	41 2	108		109	0 99
0 700	0	44 5	117		120	0 97
0 600	0	49 6	130		135	0 96
0 550	0	52 2	137		145	0 95
0 520	0 6	55 2	145	146	150	0 97
0 500	1 1	58 0	152	154	156	0 99
0 475	2 4	62 0	162	166	163	1 02
0 450	4 3	61 5	161	168	170	0 99
0 425	19 8	53 0	139	173	178	0 97
0 400	50 4	31 0	81	164	188	
0 375	79 0	9 2	24	115	198	
0 350	88 9	2 0	5		210	
verage						0 98

Volume of a perfect osmometer in per cent of its initial volume assuming the initial water :ent to be 70 per cent by volume

R value is the ratio observed cell volume (corrected for hemolysis) to the expected osmo :ic volume

Values in per cent of the initial volume of the cells in 0 9 per cent NaCl solution

)ata thus determined on a sample of normal blood are presented in table 1 umn 1 indicates the tonicities at which the readings of hemolysis listed in ımn 2 and of cell volume listed in column 3 were made In column 4 the values cell volume at the respective tonicities are converted to percentages of the initial ume as read in the 0 9 per cent NaCl solution and in column 5 these values in ı are corrected for hemolysis (employing the values in column 2) to indicate the : volume or swelling of the cells remaining intact in the tubes where partial ıolysis occurred The values for expected osmometric volume given in column 6 based on the formula of Ponder[19] $V = W(1\ T\ -1) + 100$ where $V =$ the new ume of the cells in per cent of their initial volume $W =$ the percentage by ume of water in the cells taken as 70 per cent $T =$ the ratio of the tonicity of

finding discrepancies between observed volumes and expected volumes of cells at different tonicities when some erythrocytes did not behave as perfect osmometers Where the observed swelling agrees with that calculated from the formula with the water content of the cells assumed to be 70 per cent the R value is 1 00 The R values at different points in the series range between 0 95 and 1 0., a degree of variability that may be regarded as approximately the experimental error of the method

Values for the mean volume of the cells in the undiluted heparinized blood were calculated as usual from the hematocrit measurement[8] and the cell count and the

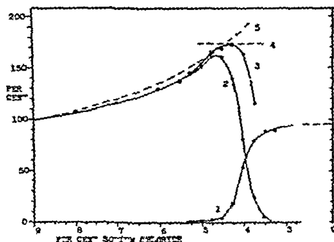


FIG 1 OSMOTIC BEHAVIOR OF NORMAL ERYTHROCYTES IN THE BLOOD OF A HEALTHY CHILD S S 3 YEARS OF AGE

Hemolysis and changes in volume of erythrocytes in hypotonic salt solutions (1) Hemolysis expressed in per cent of the total hemoglobin in the sample (2) Observed cell volume per cent of the initial volume of the cells in 0 9 per cent NaCl solution (3) Cell volumes corrected for hemolysis i.e the volume of the unhemolyzed cell (4) Expected maximal cell volume calculated from the mean surface area (5) Expected osmometric volume of the cells at different tonicities assuming the initial water content of the cells to be 70 per cent Data on this blood are listed in table 1

mean diameter of the cells was read from stained films by means of the Haden Hauser erythrocytometer [11] The mean thickness (T) was calculated from the mean volume and diameter ($T = V/\pi r^2$) The mean surface area was calculated as that of a flat disc of the same thickness and diameter The expected maximal swelling was calculated as the percentile relationship of the volume of a sphere with the same surface area to the initial mean volume of the cells i e the percentile swelling expected if the cells attain their maximal volume as spheres within the limits of their mean surface area following essentially the steps suggested by Haden [7 8]

OBSERVATIONS

Several different patterns of osmotic behavior of human erythrocytes are illustrated by examples cited in the accompanying tables and figures

TABLE 3.—*Osmotic behavior of erythrocytes of patients suffering various diseases*

Subjects	Initial Mean Dimensions				Volume of a sphere with same surface area	Tonicity	Hemolysis at this tonicity	Expected osmometric volume at this tonicity	Maximum Cell Volume		*R* value
	Volume	Diameter	Thickness	Surface area					*Expected* calculated from surface area	Found observed volume corrected for hemolysis	
Congenital spherocytosis											
	μ³	μ	μ	μ	μ³	% NaCl	%	%*	%†	%†	
P H	85	7.0	2.21	126	133	0.500	9.0	156	156	156	1.00—Av
H A	77	6.8	2.12	118	120	0.525	1.0	150	156	154	1.00—Av
P A	85	6.4	2.64	117	119	0.575	23.0	140	140	138	0.99—Av
J J	75	6.6	2.22	114	114	0.550	16.5	145	152	152	1.01—Av
J J	77	6.4	2.39	113	112	0.600	16.6	135	145	128	0.98—Av
P J	74	6.3	2.33	108	105	0.550	44.9	145	142	138	0.97—Av
M J	79	6.2	2.62	111	110	0.600	46.7	135	139	135	1.00—Av
D J	72	6.3	2.31	108	105	0.525	13.9	150	146	148	0.98—Av
W M	82	7.0	2.13	124	130	0.525	14.3	150	159	153	1.01—Av
W M	78	7.3	1.81	125	132	0.450	43.7	170	174	168	1.01—Av
Hypochromic anemia											
F D	75	7.8	1.57	134	146	0.375	24.3	198	195	199	1.00—Av
F H	71	7.2	1.74	121	125	0.450	29.2	170	176	171	1.04—Av
B M	63	6.9	1.69	111	110	0.450	31.7	170	175	169	1.00—Av
R G	57	6.5	1.72	102	97	0.475	9.3	163		161	1.00—Av
						0.350	69.7	210	170	167	0.80
D P	55	6.8	1.51	105	101	0.450	34.0	163		165	1.02—Av
						0.400	50.0	188	184	166	0.87
L W	50	6.6	1.46	99	93	0.500	28.6	156		159	1.04—Av
						0.450	48.9	170	186	155	0.91
Sicklemia											
M J W	65	7.9	1.33	131	141	0.400	1.0	188		165	0.88—Av
						0.275	15.7	260	217	219	0.85
M W	79	8.0	1.57	140	156	0.600	1.5	135		124	0.92—Av
						0.350	42.0	210	197	156	0.74
E J	89	7.8	1.87	141	157	0.525	1.3	150		137	0.91—Av
						0.375	30.8	198	176	178	0.90
Thallassemia											
G B	94	9.1	1.45	171	210	0.500	1.0	156		138	0.88
						0.2[illegible]5	18.0	260	220	213	0.81
J B	62	7.4	1.45	120	124	0.375	21.0	198	200	199	0.98—Av
Pernicious anemia											
J W	153	9.7	2.07	211	288	0.525	1.0	150		143	0.95—Av
						0.400	33.9	188	188	167	0.89

* Volume of a perfect osmometer in per cent of its initial volume, assuming the initial water content to be 70 per cent by volume.

† Values in per cent of the initial volume of cells in 0.9 per cent NaCl solution.

R values "Av" represent the average for all points in the series up to the tonicity indicated; other values are for the one point indicated, e.g., for the patient R. G. with hypochromic anemia the average of *R* values for tonicities 0.8 to 0.475 per cent NaCl was 1.00, but at 0.350 where maximal swelling of the cells occurred, the *R* value was 0.80.

finding discrepancies between observed volumes and expected volumes of cells at different tonicities when some erythrocytes did not behave as perfect osmometers Where the observed swelling agrees with that calculated from the formula with *the water content of the cells assumed to be 70 per cent the R value is 1 00 The R* values at different points in the series range between 0 95 and 1 02 a degree of variability that may be regarded as approximately the experimental error of the method

Values for the mean volume of the cells in the undiluted heparinized blood were calculated as usual from the hematocrit measurement[6] and the cell count and the

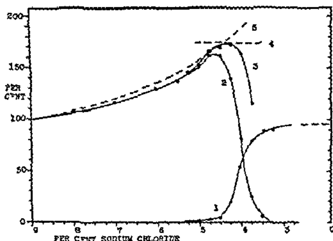


FIG 1 OSMOTIC BEHAVIOR OF NORMAL ERYTHROCYTES IN THE BLOOD OF A HEALTHY CHILD S S 3 YEARS OF AGE

Hemolysis and changes in volume of erythrocytes in hypotonic salt solutions (1) Hemolysis expressed in per cent of the total hemoglobin in the sample (2) Observed cell volume per cent of the initial volume of the cells in 0 9 per cent NaCl solution (3) Cell volumes corrected for hemolysis i e the volume of the unhemolyzed cells (4) Expected maximal cell volume calculated from the mean surface area (5) Expected osmometric volume of the cells at different tonicities assuming the initial water content of the cells to be 70 per cent Data on this blood are listed in table 1

mean diameter of the cells was read from stained films by means of the Haden Hauser erythrocytometer[11] The mean thickness (T) was calculated from the mean volume and diameter ($T = V/\pi r^2$) The mean surface area was calculated as that of a flat disc of the same thickness and diameter The expected maximal swelling was calculated as the percentile relationship of the volume of a sphere with the same surface area to the initial mean volume of the cells i e the percentile swelling expected if the cells attain their maximal volume as spheres within the limits of their mean surface area following essentially the steps suggested by Haden[7,8]

OBSERVATIONS

Several different patterns of osmotic behavior of human erythrocytes are illustrated by examples cited in the accompanying tables and figures which are se

TABLE 3 —*Osmotic behavior of erythrocytes of patients suffering various diseases*

Subjects	Initial Mean Dimensions				Volume of a sphere with same surface area	Tonicity	Hemolysis at this tonicity	Expected osmometric volume at this tonicity	Maximum Cell Volume		*R* value
	Volume	Diameter	Thickness	Surface area					*Expected* calculated from surface area	*Found* observed volume corrected for hemolysis	
Congenital spherocytosis											
	μ	μ	μ	μ	μ	% NaCl	%	%	%†	%†	
P H	85	7 0	2 21	126	133	0 500	9 0	156	156	156	1 00—Av
H A	77	6 8	2 12	118	120	0 525	1 0	150	156	154	1 00—Av
P A	85	6 4	2 64	117	119	0 575	23 0	140	140	138	0 99—Av
J J	75	6 6	2 22	114	114	0 550	16 5	145	152	152	1 01—Av
J J	77	6 4	2 39	113	112	0 600	16 6	135	145	128	0 98—Av
P J	74	6 3	2 33	108	105	0 550	44 9	145	142	138	0 97—Av
M J	79	6 2	2 62	111	110	0 600	46 7	135	139	135	1 00—Av
D J	72	6 3	2 31	108	105	0 525	13 9	150	146	148	0 98—Av
W M	82	7 0	2 13	124	130	0 525	14 3	150	159	153	1 02—Av
W M	78	7 3	1 81	125	132	0 450	43 7	170	174	168	1 01—Av
Hypochromic anemia											
F D	75	7 8	1 57	134	146	0 375	24 3	198	195	199	1 00—Av
F H	71	7 2	1 74	121	125	0 450	29 2	170	176	171	1 04—Av
B M	63	6 9	1 69	111	110	0 450	31 7	170	175	169	1 00—Av
R G	57	6 5	1 72	102	97	0 475	9 3	163		161	1 00—Av
						0 350	69 7	210	170	167	0 80
D P	55	6 8	1 51	105	101	0 450	34 0	163		165	1 02—Av
						0 400	50 0	188	184	166	0 87
L W	50	6 6	1 46	99	93	0 500	28 6	156		159	1 04—Av
						0 450	48 9	170	186	155	0 91
Sicklemia											
M J W	65	7 9	1 33	131	141	0 400	1 0	188		165	0 88—Av
						0 275	15 7	260	217	219	0 85
M W	79	8 0	1 57	140	156	0 600	1 5	135		124	0 92—Av
						0 350	42 0	210	197	156	0 74
E J	89	7 8	1 87	141	157	0 525	1 3	150		137	0 91—Av
						0 375	30 8	198	176	178	0 90
Thallassemia											
G B	94	9 1	1 45	171	210	0 500	1 0	156		138	0 88
						0 275	18 0	260	220	213	0 81
J B	62	7 4	1 45	120	124	0 375	21 0	198	200	199	0 98—Av
Pernicious anemia											
J W	153	9 7	2 07	211	288	0 525	1 0	150		143	0 95—Av
						0 400	33 9	188	188	167	0 89

Volume of a perfect osmometer in per cent of its initial volume assuming the initial water content to be 70 per cent by volume

† Values in per cent of the initial volume of cells in 0 9 per cent NaCl solution

R values Av represent the average for all points in the series up to the tonicity indicated other values are for the one point indicated e g for the patient R G with hypochromic anemia the average of *R* values for tonicities 0 8 to 0 475 per cent NaCl was 1 00 but at 0 350 where maximal swelling of the cells occurred the *R* value was 0 80

of beginning hemolysis (curve 1) followed closely the expected osmometric volume (curve 5) at each tonicity to reach exactly the expected maximal cell volume (horizontal line 4) predicted from the mean surface area calculated from the measurements of initial dimensions

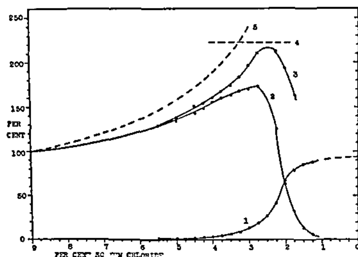


Fig 4 Thalassemia (Cooley s Disease or Mediterranean Anemia) in a Boy G B 15 Years of Age

For explanation of symbols see figure 1

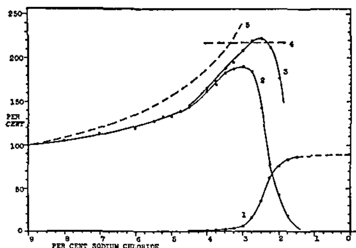


Fig 5 Sicklemia in a Negro Boy M J W 10 Years of Age

For explanation of symbols see figure 1

In table 2 data on the erythrocytes of normal subjects adults children and new born infants are arranged to show how normal cells though varying considerably in their dimensions of mean volume diameter and thickness conform closely to the pattern of behavior illustrated in figure 1 Normal erythrocytes usually attain a maximal volume of from 170 to 175 per cent of their initial volume and this degree

lected from a large series of studies that were made during the years 1939 to 1943 with the assistance of Miss Mary Wing Presumably normal red cells from healthy

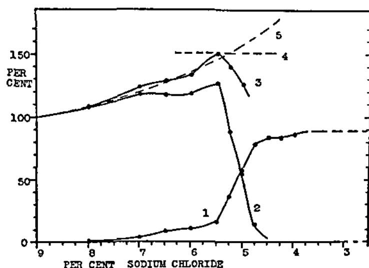


FIG 2 CONGENITAL SPHEROCYTOSIS OR HEMOLYTIC ICTERUS IN A BOY J J 4 YEARS OF AGE

For explanation of symbols see figure 1

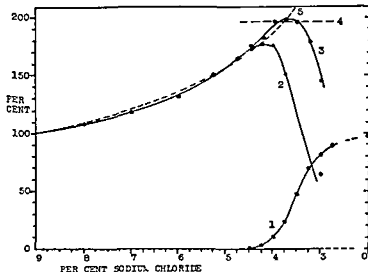


FIG 3 MILD ANEMIA WITH HYPOCHROMIA AND MICROCYTOSIS PRESUMABLY DUE TO SIMPLE IRON DEFICIENCY IN A WOMAN F D

For explanation of symbols see figure 1

subjects are represented in tables 1 and 2 and figure 1 abnormal types of cells are represented in table 3 and in figures 2 to 7

Normal subjects Observations on the blood of a normal child S S are illustrated by figure 1 and by data listed in table 1 and in table 2 example 17 In the figure curves 2 and 3 show how the swelling of the cells before and after the point

of beginning hemolysis (curve 1) followed closely the expected osmometric volume (curve 5) at each tonicity to reach exactly the expected maximal cell volume (horizontal line 4) predicted from the mean surface area calculated from the *measurements of initial dimensions*

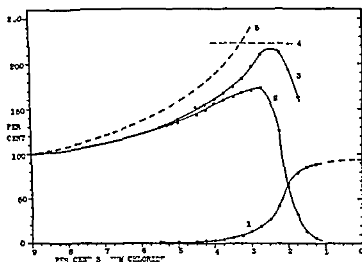


Fig 4 Thalassemia (Cooley's Disease or Mediterranean Anemia) in a Boy G B 15 Years of Age

For explanation of symbols see figure 1

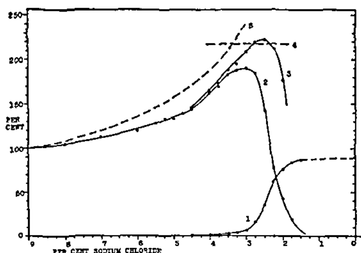


Fig 5 Sicklemia in a Negro Boy M J W 10 Years of Age

For explanation of symbols see figure 1

In table 2 data on the erythrocytes of normal subjects adults children and new born infants are arranged to show how normal cells though varying considerably in their dimensions of mean volume diameter and thickness conform closely to the pattern of behavior illustrated in figure 1 Normal erythrocytes usually attain a maximal volume of from 170 to 175 per cent of their initial volume and this degree

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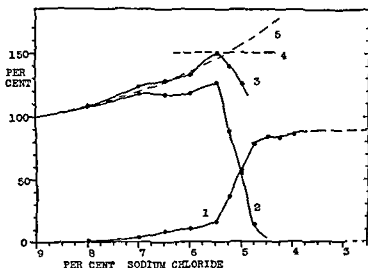


Fig 2 Congenital Spherocytosis or Hemolytic Icterus in a Boy J J 4 Years of Age

For explanation of symbols see figure 1

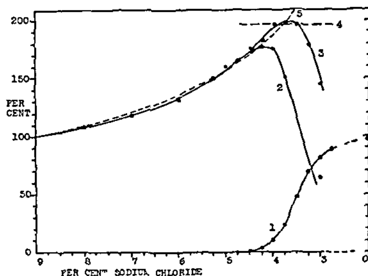


Fig 3 Mild Anemia with Hypochromia and Microcytosis Presumably Due to Simple Iron Deficiency in a Woman F D

For explanation of symbols see figure 1

subjects are represented in tables 1 and 2 and figure 1 abnormal types of cells are represented in table 3 and in figures 2 to 7

Normal subjects Observations on the blood of a normal child S S are illustrated by figure 1 and by data listed in table 1 and in table 2 example 17 In the figure curves 2 and 3 show how the swelling of the cells before and after the point

Congenital spherocytosis Figure 2 illustrates diagramatically the osmometric behavior of the erythrocytes of a 4 year old boy (sample J J in table 3) suffering typical manifestations of congenital hemolytic jaundice Hemolysis of these cells began in 0 8 per cent NaCl solution and appeared to be complete with cellular residue too low to be read in 0 45 per cent solution (although the hemoglobin liberated in this solution represented only 85 per cent hemolysis) The swelling of the unhemolyzed cells followed fairly closely the expected osmometric volume and reached exactly the calculated maximum volume 152 per cent of the initial cell volume

In table 3 are listed data on ten samples of bloods from patients suffering congenital hemolytic jaundice Hemolysis of the cells in these bloods began in solutions of NaCl varying from 0 85 to 0 65 per cent and in all instances appeared to be complete (by visual inspection) in solutions around 0 40 per cent Except for the last sample listed the maximum swelling of these erythrocytes was between 128 and 156 per cent of their initial volume The last two samples listed from the patient W M were drawn respectively before and two weeks after splenectomy A great improvement in the patient s general condition was observed after splenectomy The postoperative blood sample showed lessened red-cell fragility as indicated by the point of beginning hemolysis and by greater maximum swelling compared with that of the sample drawn before operation In all instances the maximum swelling was predicted fairly closely from the mean surface area of the cells and the R values were about the same (within limits of experimental error) as those of normal erythrocytes at all tonicities up to the point of maximal swelling

Hypochromic anemia In studies on patients with varying degrees of anemia presumed to be due to simple iron deficiency different patterns of osmotic behavior of the red cells were found among bloods showing varying degrees of hypochromia and microcytosis The first example in this group cited in table 3 and figure 3 was a blood sample from a Negro woman F D with mild anemia with hemoglobin concentrations in the whole blood 9 9 grams and in the packed cells 30 grams per 100 cc and the mean cell volume 75 cubic microns The unusually high value for maximal swelling of these cells 199 per cent of their initial volume agreed closely with the predicted value and the swelling followed very closely the expected osmometric swelling up to the maximal point with an average R value 1 00 In the last three examples cited in this group R G D P L W with cells showing a greater degree of microcytosis and hypochromia the swelling of the cells followed the expected osmometric volumes up to a certain point in each case after the beginning of hemolysis but thereafter the swelling of the remaining cells was less than expected (R values at first 1 00 then 0 80 to 0 91) and the maximum volumes attained were less than the values predicted from the mean surface area

Thalassemia The first of the two sets of data listed under thalassemia in table 3 and illustrated in figure 4 concern the erythrocytes of a 15 year old boy G B who exhibited typical manifestations of Mediterranean or Cooley s anemia with large spleen moderate skeletal changes moderate anemia and normoblastemia The red cells were moderately large in mean volume and diameter but thinner than normal There was the usual wide span of osmotic resistance with hemolysis beginning at

of swelling is found most often in about 0 425 per cent NaCl solution The R values in table 2 represent the average of all values determined in the series for each blood up to the point of maximal swelling Among the bloods from adults and

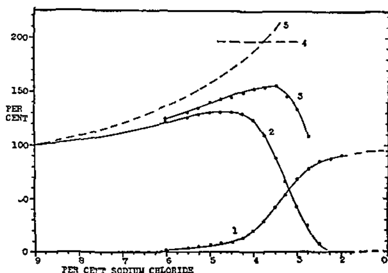


FIG 6 SICKLEMIA WITH ANEMIA IN A NEGRO BOY M W 4 YEARS OF AGE

For explanation of symbols see figure 1

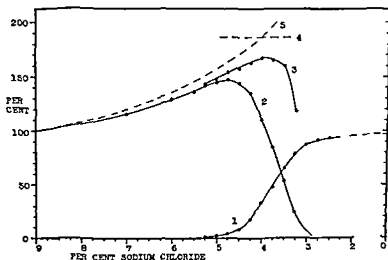


FIG 7 PERNICIOUS ANEMIA IN A MAN O YEARS OF AGE WITH RETICULOCYTOSIS SHORTLY AFTER TREATMENT WITH LIVER EXTRACT

For explanation of symbols see figure 1

children listed the deviations of the *R* values from the theoretically expected value of 1 00 are scarcely more than can be ascribed to experimental error It is to be noted that the *R* values for the macrocytic cells of newborn infants were consistently slightly low around 0 95 but the observed maximal swelling agreed closely with the values around 165 per cent predicted from the mean surface area

Congenital spherocytosis Figure 2 illustrates diagramatically the osmometric behavior of the erythrocytes of a 4 year old boy (sample J J in table 3) suffering typical manifestations of congenital hemolytic jaundice Hemolysis of these cells began in 0 8 per cent NaCl solution and appeared to be complete with cellular residue too low to be read in 0 45 per cent solution (although the hemoglobin liberated in this solution represented only 85 per cent hemolysis) The swelling of the unhemolyzed cells followed fairly closely the expected osmometric volume and reached exactly the calculated maximum volume 152 per cent of the initial cell volume

In table 3 are listed data on ten samples of bloods from patients suffering congenital hemolytic jaundice Hemolysis of the cells in these bloods began in solutions of NaCl varying from 0 85 to 0 65 per cent and in all instances appeared to be complete (by visual inspection) in solutions around 0 40 per cent Except for the last sample listed the maximum swelling of these erythrocytes was between 128 and 156 per cent of their initial volume The last two samples listed from the patient W M were drawn respectively before and two weeks after splenectomy A great improvement in the patient s general condition was observed after splenectomy The postoperative blood sample showed lessened red cell fragility as indicated by the point of beginning hemolysis and by greater maximum swelling compared with that of the sample drawn before operation In all instances the maximum swelling was predicted fairly closely from the mean surface area of the cells and the R values were about the same (within limits of experimental error) as those of normal erythrocytes at all tonicities up to the point of maximal swelling

Hypochromic anemia In studies on patients with varying degrees of anemia presumed to be due to simple iron deficiency different patterns of osmotic behavior of the red cells were found among bloods showing varying degrees of hypochromia and microcytosis The first example in this group cited in table 3 and figure 3 was a blood sample from a Negro woman F D with mild anemia with hemoglobin concentrations in the whole blood 9 9 grams and in the packed cells 30 grams per 100 cc and the mean cell volume 75 cubic microns The unusually high value for maximal swelling of these cells 199 per cent of their initial volume agreed closely with the predicted value and the swelling followed very closely the expected osmometric swelling up to the maximal point with an average R value 1 00 In the last three examples cited in this group R G D P L W with cells showing a greater degree of microcytosis and hypochromia the swelling of the cells followed the expected osmometric volumes up to a certain point in each case after the beginning of hemolysis but thereafter the swelling of the remaining cells was less than expected (R values at first 1 00 then 0 80 to 0 91) and the maximum volumes attained were less than the values predicted from the mean surface area

Thalassemia The first of the two sets of data listed under thalassemia in table 3 and illustrated in figure 4 concern the erythrocytes of a 15 year old boy G B who exhibited typical manifestations of Mediterranean or Cooley s anemia with large spleen moderate skeletal changes moderate anemia and normoblastemia The red cells were moderately large in mean volume and diameter but thinner than normal There was the usual wide span of osmotic resistance with hemolysis beginning at

0 5 and not complete until around 0 1 per cent NaCl At all tonicities the observed swelling was less than the expected osmometric swelling with the R value gradually diminishing from 0 88 to 0 81 but the final maximal volume of 213 per cent approached closely the value predicted from the mean surface area The second set of data listed in the table relate to this boy s brother J B who showed no physical manifestations of this disorder but whose blood showed the typical target cells and microcytosis such as have been noted by several investigators[25b] among essentially healthy members of the families of patients suffering with Cooley s disease These cells were small and thin they too had a rather wide span of resistance but their swelling followed closely the normal expected pattern up to a maximum volume of 199 per cent with behavior like that observed in the case of F D illustrated in figure 3 with mild hypochromic microcytic anemia Two other siblings in this family had mild anemia that did not respond to the administration of iron with microcytic hypochromic erythrocytes and target cells like those of the blood of J B and displaying similar osmotic behavior Apparently the defect responsible for poor osmometric behavior in the cells of the patient G B was not present in the cells of the siblings although the microcytes and target cells in such families have been regarded as part of the genetic picture of thalassemia

Sicklemia The two patients M J W and M W represented in figures 5 and 6 respectively displayed noteworthy differences in symptomatology as well as differences in the osmotic behavior of their red cells The first M J W represented in figure 5 had experienced little illness was not known to have had crises of anemia or jaundice at any time and the spleen was not palpable Sickling of his cells in a sealed cover glass preparation occurred quickly and involved practically 100 per cent of the cells The other represented in figure 6 had suffered repeated crises of abdominal pain associated with anemia slight jaundice and enlargement of the spleen Sickling in his blood occurred rather slowly and involved about half of the cells in a sealed cover glass preparation allowed to stand twenty four hours at room temperature Reticulocytes in the two bloods were respectively 2 6 and 11 2 per cent The erythrocytes of both bloods were thinner than normal (see data in table 3) The swelling of the cells in both bloods at all tonicities was considerably less than the expected osmometric swelling The cells represented in figure 5 attained a maximum volume close to that predicted from the surface area but in figure 6 the observed maximal volume is seen falling far below the predicted value Figure 5 shows a considerably increased osmotic resistance with only slight hemolysis appearing at tonicities from 0 45 to 0 35 per cent NaCl and complete hemolysis at around 0 125 per cent Figure 6 shows a diminished resistance of part of the cells with hemolysis beginning in 0 6 per cent NaCl solution and complete at around 0 225 per cent Data on the red cells of a third patient (subject E J) with sicklemia cited in table 3 resemble those of the subject M J W in that the R values were consistently low but the observed maximal volume agreed closely with the predicted maximal volume

Pernicious anemia Observations on the erythrocytes of a patient J W suffering from pernicious anemia are illustrated in figure 7 other data on these cells are

listed in table 3 This sample of blood was obtained when the reticulocyte count was 25 per cent following the administration of liver extract The erythrocytes were typically large in all dimensions their swelling at all tonicities was less than the expected osmometric swelling and the maximal volume found was considerably less than predicted from the mean surface area These observations are presented not to suggest that such findings are necessarily typical of pernicious anemia (further observations on other patients are lacking) but to illustrate again a pattern of abnormal behavior found in red cells of pathologic type

Miscellaneous Normal osmotic behavior of erythrocytes corresponding closely to that illustrated in figure 1 has been found in cases of polycythemia vera cirrhosis of the liver anemia associated with acute and chronic nephritis lipoid nephrosis and other metabolic diseases

Discussion

These observations indicate that normal human erythrocytes and also some abnormal types of red cells varying considerably in size shape and hemoglobin content display a remarkably uniform pattern of osmotic behavior in hypotonic salt solutions swelling like perfect osmometers up to a maximum volume of spheres within the limits of their surface areas Contrasting pictures may be seen in studies of the thick cells spherocytes of congenital hemolytic icterus and the thin cells of mild hypochromic anemia as illustrated in figures 2 and 3 respectively The patterns of behavior exhibited by the two types of cells appear to be governed by fundamentally similar principles although their relative fragility appears to be quite different The abnormal fragility of the cells of congenital hemolytic icterus probably depends on the character of the internal structure of the cells that is responsible for their greater than normal thickness rather than on any abnormality of the surface membrane affecting its permeability or susceptibility to stress

The well known increased resistance or decreased fragility of the red cells of thalassemia indicated by the points of beginning and complete hemolysis in the Hamburger series can be explained by two mechanisms demonstrated in figure 4 First the cells behave as poor osmometers swelling less than expected at all tonicities and second the very thin cells of large diameter are capable of much greater percentile swelling than normal cells within the limits of their surface area Such explanation applies also to the somewhat increased osmotic resistance of the cells of the patient with sicklemia represented in figure 5

The red cells in all three cases of sicklemia cited in table 3 behaved as poor osmometers swelling less than expected at all tonicities The span of hemolysis was wide in each instance In two cases the observed maximal swelling of the cells agreed closely with the value predicted from the mean surface area but in the case of M W (fig 6) the observed maximal swelling was much less than predicted It seems possible that the patients represent distinctly different clinical types one showing merely the sickling trait with increased resistance of the cells, and the other a more complicated picture of a hemolytic type of anemia with increased fragility of at least a part of the red cells The patient M W displayed

symptoms and physical manifestations with crises of jaundice anemia and enlargement of the spleen compatible with this concept There is of course the possibility that a hemolytic trait may be superimposed on the sickling trait

Investigators who have found erythrocytes swelling less in hypotonic fluids than should be expected if they imbibe water like perfect osmometers have offered several explanations for this behavior namely (1) that salts escape from the cells in the course of their swelling not necessarily at a constant rate nor reaching the same equilibria at different tonicities (2) that variations in the water content of the cells may be large, so that the formula for osmometric swelling based on an assumed initial water content of 70 per cent cannot be applied to all erythrocytes alike (3) that some of the water in the cells is bound to hemoglobin and other constituents and is not transferrable by osmosis (4) that phenomena of gelation in some cells may lead to physical conditions quite unlike those assumed for a simple sac or envelope containing hemoglobin and other osmotically active substances in solution [19, 1]

It would appear that any one or several of these explanations might apply to the osmotic behavior of abnormal types of erythrocytes such as are cited here It is well to note with regard to seemingly contradictory statements of various investigators regarding the osmotic behavior of the erythrocyte that generalizations are not sound when they imply (as some have) that observations on the red cells of one or a few members of a species can be applied to all individuals of the species or to other species It must be borne in mind that erythrocytes of different species differ considerably in their characteristics of osmotic fragility also that differences in osmotic behavior of red cells drawn from the same individual at different times may arise from alterations in cellular composition and metabolic activities induced by various pathologic conditions which may not be readily apparent and may even be transient *

Summary

Normal human erythrocytes suspended in the series of hypotonic salt solutions employed for testing red cell fragility behave like nearly perfect osmometers throughout the series with their maximum swelling usually around 175 per cent of their initial volume sharply defined by their mean surface area The same principles govern the swelling and hemolysis of some abnormal types of erythrocytes with *different characteristics of abnormal fragility or resistance to osmotic hemolysis* The red cells of congenital hemolytic icterus exhibit essentially normal osmotic behavior but since the spherocytes can swell very little within the limits of their surface area they rupture at higher tonicities with the maximal swelling in most instances around 150 per cent of their initial volume Thin cells are capable of greater swelling than normal cells Moderately hypochromic erythrocytes from patients with mild anemia behaved like perfect osmometers throughout the series

* The effects of environmental factors e g anticoagulants diluting fluids time of preservation temperature etc on erythrocytes prior to studies of their osmotic behavior have been thoroughly discussed in several papers of the symposium on blood preservation recently published in the J Clin Investigation 26 591–755 July 194

to attain a maximal swelling of around 200 per cent this value agreeing with predictions based on their mean surface area

Erythrocytes of patients with thalassemia sicklemia and pernicious anemia exhibited less than the expected osmometric swelling throughout the series of hypotonic solutions The cells behaving as imperfect osmometers displayed varying patterns of hemolysis and of maximal swelling in relation to predicted values The increased osmotic resistance characteristic of the cells of thalassemia is accounted for by two mechanisms they swell less than normal cells at each tonicity and being thin they undergo greater swelling (around 220 per cent) before they are hemolyzed

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Summary

Normal human erythrocytes suspended in the series of hypotonic salt solutions employed for testing red cell fragility behave like nearly perfect osmometers throughout the series with their maximum swelling usually around 175 per cent of their initial volume sharply defined by their mean surface area The same principles govern the swelling and hemolysis of some abnormal types of erythrocytes with different characteristics of abnormal fragility or resistance to osmotic hemolysis The red cells of congenital hemolytic icterus exhibit essentially normal osmotic behavior but since the spherocytes can swell very little within the limits of their surface area they rupture at higher tonicities with the maximal swelling in most instances around 150 per cent of their initial volume Thin cells are capable of greater swelling than normal cells Moderately hypochromic erythrocytes from patients with mild anemia behaved like perfect osmometers throughout the series

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A METHOD FOR DETERMINING THE FORM OF THE DISTRIBUTION OF RED CELL RESISTANCES TO SIMPLE HEMOLYSINS

By Eric Ponder M D D Sc

THE DETERMINATION of the way in which the resistances of red cells are distributed with respect to hypotonicity (the erythrocyte fragility test) is a familiar laboratory procedure and methods have been described for finding the distribution of the resistances of red cells to heat [1] to acid [2] to shaking [4 5 6] to saponin hemolysis[7 8] and to hemolysis by lysolecithin [9] It is now recognized that the form of the frequency distribution of red cell resistances to hypotonicity is determined primarily by the variations in the shape of the cells of the system [10–14] and it is reasonable to think that the forms of the frequency distributions of resistances to lysins such as saponin or digitonin depend on the chemical nature and spatial arrangement of the components of the red cell architecture i e on the consist * of the cell or of its surface ultrastructure Just as it is necessary to have a method by means of which fragility can be described quantitatively before its relation to shape can be appreciated so it is necessary to have a method by means of which the distribution of resistances to lysins can be described quantitatively before it is possible to study the relation of the resistance to chemical composition spatial arrangement etc

The purpose of this paper is to describe a method for measuring the resistance of red cells to simple hemolysins to define the range of normal variation and to give a few illustrations of abnormal variations in resistance which occur under conditions in which it is reasonable to believe that abnormal variations in the consist of the members of the cell population have occurred Emphasis will be placed on the observations being made and treated so that the maximum amount of information can be extracted from them because the full potentialities of methods for determining red cell resistances are not realized when they are carried out in simplified form Further it should be remarked at the outset that the method to be described is of general application although it is illustrated by results obtained with only two simple hemolysins in addition to the results of fragility determinations

1 Composition of the Hemolytic Systems

To illustrate the procedure the t o lysins saponin and digitonin ha e been selected for several reasons More is known about the kinetics of lysis by saponin than by any other lysin and the flat form of the frequency d stribution of resistances makes it particularly usef l for picking up bimodalities etc Digitonin is selected beca e it is one of the few powerf l lysins which can be obtained in the pu e state (unlike the saponins the activity of hich is variable) and free from inhibitory contaminants (unl ke most preparations of the bile salts)

(*a*) *Saponin* Add 10 mg of quillia saponin (British Dr g Houses) to 100 ml of 1 per cent NaCl

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From The Nassau Ho pital Mineola N Y

This is noun the general use of which has been u ged by Schlegel [7] it is employed in describing the composition of railv ay trains Its definition is Consist *n* The singular elements of which something is constituted together with all the relevant spatial arrangements of these elements As Schlegel points out there is no other word in our language which conveys quite the same idea

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baths but 25 C is a convenient temperature which can easily be maintained to within ±1 C for five hours at a time in the average laboratory room

The hemolytic systems are mixed either by rotary shaking or by inversion e ery fifteen minutes and the amount of hemolysis in each is determined at the end of five hours

2 Measurement of Percentage Hemolysis

To find the percentage of complete hemolysis present in any one of the hemolytic systems at the end of five hours at 25 C the cells are thrown down by spinning for a few minutes at 2000 r p m The supernatant fluid can then be poured off into a dry vial without disturbing the cells Two ml of this supernatant fluid is added to 50 ml of distilled water and the optical density is measured at a wave length of about 4500 Å (blue filter) with a Lumetron photometer or with any other photometer of a similar type This measurement is converted into a value for percentage

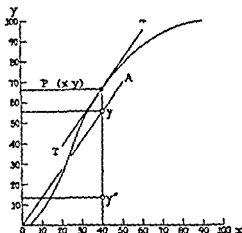


Fig 1 Diagram to Illustrate the Method of Graphic Differentiation
For description see text

hemolysis by referring to a calibration curve prepared by measuring the optical densities corresponding to 100 50 25 and 12 5 per cent of complete hemolysis of the cells contained in the hemolytic systems When measured at 4500 A the optical density is so related to the concentration of Hb present that percentages of complete hemolysis are found with about the same degree of accuracy over the whole percentage hemolysis range

A separate calibration curve must be prepared for each suspension used in practice one uses the pooled contents of several completely hemolyzed systems for obtaining the 100 per cent point and finds the other points by successive dilutions by powers of 2.

3 Plotting and Differentiation of the Curves

The values of percentage hemolysis P are plotted against the quantity of lysin in γ contained in the system using the same scales for ordinates and abscissae as those used in figures 2 3 and 4 The choice of the scales is important as it de

this gives a 1 in 10 000 or a 200γ/2 ml solution of the lysin A series of dilutions is made from this so that 100 ml volumes of a series of solutions of saponin containing 100 80 60 50 40 30 25 20 15 12 10 8 6 4 3 2 and 1 γ per 2 ml are obtained The solutions are stored in stoppered bottles at temperature 4 C and keep for about a month

(*b*) *Digitonin* 100 ml volumes of solutions of digitonin (Merck) in 1 per cent NaCl and containing 20 17 5 15 12 5 11 25 10 8 75 7 5 6 25 5 0 3 75 2 5 and 1 25 γ per 2 ml are prepared Because of the steepness of the digitonin percentage hemolysis curve intermediate concentrations e g 8 125 γ/2 ml may be needed if so 2 ml of such a concentration can be made by mixing 1 ml of the concentrations above it and below it in the series i e 8 75γ/2 ml and 7 5γ/2 ml As the final values for hemolysis are reached within fifteen to thirty minutes in systems containing digitonin the need for making additional observations at such intermediate concentrations can be appreciated soon after the experiment is set up systems containing the intermediate concentrations can then be added if necessary and the results can still be read at the end of 5 hours without any error having been introduced The digitonin solutions keep at 4 C for about a month

(*c*) *Hypotonic NaCl* A series of solutions of hypotonic NaCl is prepared * starting with a 0 5 per cent NaCl and descending by a common difference of 0 02 units i e 0 50 0 48 0 46 per cent NaCl in the scale of tonicity (the tonicity of a 1 per cent NaCl corresponds to 1 tonicity unit) The lowest member of the series should be 0 14 per cent NaCl These solutions are stored at 4 C When 0 5 ml of a red cell suspension with a tonicity of 1 0 is added to 2 ml of each member of the series we get a new series for the final tonicity T_1 of the mixture this is

$$T_1 = \frac{2T_0 + 0.5}{2.5}$$

where T_0 is the tonicity of the member of the first series The common difference on the new series for the final tonicity is 0 016 so that the new series runs 0 60 0 584 0 568 with a lowest member of 0 312 per cent NaCl or tonicity units For convenience in differentiating the experimental curves the members of the new series can be described by integers starting with 0 60 = 0 so that 0 584 = 1 0 568 = 2 0 312 = 18 as in the subsidiary scale on the abscissa of the curve marked T in figure 2

(*d*) *Cell suspensions* The volume concentration ρ of the red cells in freshly drawn heparinized blood is found with a high speed hematocrit and the cells of 2 5 (0 4/ρ) ml of blood after being washed three times with 1 per cent NaCl are finally suspended in 25 ml of NaCl buffer at pH 7.0 †

(*e*) *The hemolytic systems* The hemolytic systems contained in three series of 100 x 13 mm tubes are prepared by adding 0 5 ml of the red cell suspension to 2 ml volumes of the various concentrations of saponin digitonin and hypotonic NaCl The lysins are put into the tubes first and allowed to reach the temperature at which the determinations are to be made 0 5 ml of suspension is then added to each tube with immediate shaking to produce mixing Any desired temperature can be obtained by using water

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The NaCl used must be silver free as most C P preparations now are It must also be dry Commercial preparations may contain as much as 10 per cent of water after standing around the laboratory they should be dried for twenty four hours at 120 C before use

† The NaCl buffer is made by mixing 75 ml of 1 2 Gm /100 ml NaCl with 25 ml of a mixed buffer composed of 2 ml of M 15 Na_2HPO_4 and 28 ml of M 15 NaH_2PO_4 The depression of freezing point of this NaCl buffer is the same as that of 1 Gm 100 ml NaCl (i e its tonicity is 1 0) and its pH is 7 0 at 25 C

The pH of the systems requires to be controlled at a known value because the activity of many lysins e g saponin and the bile salts has a marked pH dependence It is usual to make measurements of fragility in systems containing unbuffered hypotonic NaCl on the grounds that the situation may be complicated by the addition of the ions in the buffer mixture This is true but the situation is also liable to be complicated by uncontrolled variation in pH especially if the red cell suspension is dilute If the suspension is more concentrated the effects may be of much less [2] but since a complete investigation of the distributions of resistances to simple lysins and to hypotonic ity would include measurement of resistances at a variety of pH s one may as well buffer the system from the start

dy/dx at the point P is $y'' = y'/x$ Proceeding along the sigmoid curve systematically, tangents and their parallels through the origin are drawn with the aid of rulers fixed so as to move in parallel these can be purchased from any dealer in draughtsman s materials The value of y' (read off on the ordinate) at which the parallel through the origin cuts the ordinate of the point under consideration is noted and is divided by the value for the x coordinate of the point (read off on the

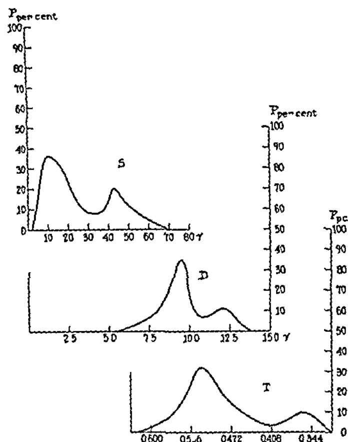


FIG 3 FREQUENCY DISTRIBUTIONS AND THEIR INTEGRALS FOLLOWING MASSIVE HEMORRHAGE

Ordinates abscissae etc as in fig 1

abscissa) the result of the division is a value of y'' the ordinate of the corresponding point on the differential curve To change the scale on which the differential curve is plotted the value of y can be divided not by x but by x/s where s is a convenient scaling constant the values $s = 10$ for saponin $s = 1$ for digitonin and $s = 2$ for hypotonic NaCl are convenient because the frequency distributions are then plotted on such a scale that their shape and variations in it can be easily appreciated by eye These values have been used in constructing the differential curves in figures 2 3 and 4 If $s = 1$ were used for saponin for example the differential curve would be so flat that variations in its shape would not be apparent on simple inspection

termines the ease and accuracy with which differentiation can be carried out Smooth sigmoid curves are drawn through the experim ntal points a possible error of about ±2 per cent being allowed in the case of each If inspection shows that the curve is bimodal or polymodal the procedure is still to draw the smoothest curve through the experimental points It will be obvious that the more points

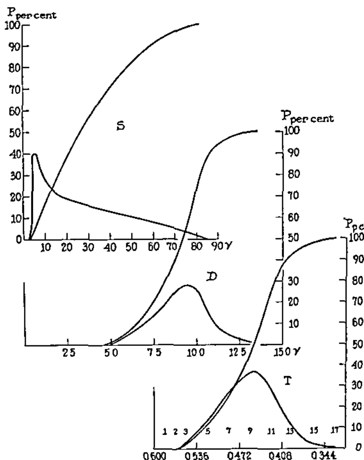


FIG 1 NORMAL FREQUENCY DISTRIBUTIONS OF RESISTANCES TOGETHER WITH THEIR INTEGRALS (THE EXPERIMENTAL CURVES)

Ordinates percentage of complete hemolysis abscissae quantity of lysin in system in γ except in curve *T* where the abscis a ho the ton city in tonicity units Subsidiary scale on abscissa of cur e *T* is that used in differentiating Curve *S* re ults for saponin curve *D* results for digitonin cur e *T* results for hypotonic NaCl Reduction 2X

there are the more certainly can the course of the curve be determined and in some cases of suspected polymodality it may be necessary to repeat the entire set of determinations *with the addition of new and strategically placed concentrations* of the lysin

The sigmoid curve is now differentiated graphically by using the following principle If TT′ is the tangent at a point P with coordinates x and y (fig 1) and if OA the parallel through the origin cuts the ordinate of P at y the value of

scatter and the skewness has been calculated from the deciles D_1 and D_2 and the median M as

$$\frac{(D_2 - M) - (M - D_1)}{(D_2 - M) + (M - D_1)}$$

by analogy with Bowley's measure of skewness The three distribution integrals with average values are plotted together with the distributions obtained from them by differentiation in figure 2

The distribution of resistances to saponin is normally negatively skew with a long tail spreading out towards its upper extreme The resistance distribution to digitonin is usually positively skew and its scatter is relatively small The normal

TABLE 1

	Average	Lowest	Highest
Saponin			
Median	26 5	22 0	28 5
Lower extreme	3 5	3 0	4 5
Upper extreme	85 0	73 0	96 0
Inter-decile diff	52 5	44 0	57 5
Skewness	−0 30	−0 15	−0 41
Digitonin			
Median	9 20	9 05	9 45
Lower extreme	4 70	3 80	5 35
Upper extreme	13 5	12 5	14 9
Inter-decile diff	4 25	4 05	4 50
Skewness	0 29	0 05	0 36
Hypotonicity			
Median	0 448	0 424	0 472
Lower extreme	0 566	0 584	0 552
Upper extreme	0 318	0 360	0 312
Inter-decile diff	0 131	0 103	0 139
Skewness	0 14	−0 06	0 22

distribution of resistances to hypotonic NaCl at pH 7 0 is usually symmetrical although it may have a small skewness in either direction None of the observed distributions shows any great individual variation from the average distribution obtained for normal red cells

5 DISCUSSION WITH EXAMPLES

The frequency distributions obtained experimentally with each lysin represent the distribution of the resistances of the N cells in the general circulation at the time the blood is drawn This number is maintained at a constant level in the normal individual by the addition of new cells at a rate P and by the removal of old cells at a rate Q so that $dN/dt = P - Q$ Changes in the rates of production and destruction result in changes in the value of N accompanied by *transient* changes in the form of

This process of differentiation can be carried out quickly and easily and is all done on the same piece of graph paper The number of tangents which require to be drawn depends to some extent on the shape of the sigmoid curve but one should be drawn at each experimental point for a start The process is completed by drawing a smooth curve through the points y'' obtained by differentiation In each case the result is a frequency curve showing the distribution of red cell resistances to the

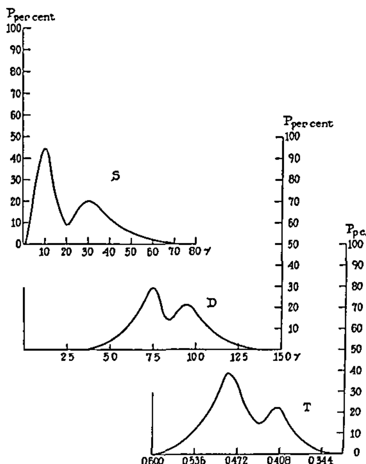


FIG 4 FREQUENCY DISTRIBUTIONS AND THEIR INTEGRALS FOLLOWING A HEMOLYTIC EPISODE

Ordinates abscissae etc as in fig 1

lysin on an abscissa showing the amount of lysin in the system in γ (or the tonicity of the system in tonicity units in the case of hypotonic NaCl)

4 NORMAL VALUES

To explore the range of normal variation the frequency distributions of resistances to saponin digitonin and hypotonic NaCl have been obtained at 25 C for the red cells of 12 normal blood donors Table 1 shows the average lowest and highest values found for various statistics of the curves The interdecile difference between the lowest and highest deciles D_1 and D has been used as a measure of

Example 3 Hodgkin's disease Red cells 3 9 millions Hb 10.4 Gm Saponin distribution median *15γ* lower extreme *2γ* upper extreme *10γ* skewness −*0 60* Digitonin distribution median *8 0γ* lower extreme *3 5γ* upper extreme *12 5γ* Hypotonic NaCl distribution median 0 456 lower extreme *0 636* upper extreme *0 294* interdecile difference *0 233*

Example 4 Aplastic anemia Red cells 1 9 million Hb 5 2 Gm Saponin distribution median *20γ* lower extreme 4γ upper extreme *58γ* interdecile difference *40γ* skewness −0 35 Digitonin distribution median 9 1γ skewness *0 45* Hypotonic NaCl distribution no abnormalities

Example 5 Normocytic anemia due to chronic infection Red cells 3 8 millions Hb 9 2 Gm Saponin distribution median *30γ* lower extreme *2γ* upper extreme *65γ* skewness −*0 03* Distributions to digitonin and to hypotonic NaCl substantially normal

Much more data will have to be accumulated before it is possible to classify these persistent abnormalities in the form of the frequency distributions to lysins It is significant that all those which I have observed have been associated with anemia In the meantime the persistent abnormalities can be looked upon as having much the same significance that abnormal Price Jones curves would have i e as showing that the normal red cell population has been partially or wholly replaced by populations having different resistance characteristics

Summary

A method is described for determining quantitatively the form of the resistance distributions of red cell resistances to simple hemolysins The normal range of variation is given for the resistance distributions to saponin digitonin and hypotonic NaCl all at pH 7 0 and examples of departures from the normal which may take the form of bimodalities after hemorrhagic or hemolytic episodes or of more persistent changes in the characteristics of the distributions are provided

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the resistance distribution variations in the distribution of the resistances of the cell in the population *N* can however be brought about in another way The *P* cells which are added to the population *N* in each unit of time themselves constitute a population with resistances distributed according to some form of frequency distribution the same applies to the *Q* cells which are removed from the population *N* in unit time and it can be shown that in the steady state the distribution of resistances in the population *P* is the same as that in the population *Q* that the form of these distributions determines the form of the distribution in the population *N* and that if the life of the red cell in the circulation depends on its resistance to the lysin in question the *P* and *Q* distributions must be more negatively skew than the *N* distribution [15] It follows that *persistent* changes in the form of the distribution of resistances in the population *N* are reflections of persistent changes in the form of the distribution of resistances in the populations *P* and *Q* and that *transient* changes in the distribution of resistances in the population *N* are due to sudden changes in the rate at which new cells are added to it or in the form of the resistance distribution in the population *P* Sudden changes in the rate of removal of old cells or in the distribution of resistances in the population *Q* can conceivably produce similar transient changes in the population *N*

Hemorrhagic or hemolytic episodes supply the clearest instances of transient changes in the form of the distribution of resistances to saponin digitonin and hypotonic NaCl as is illustrated by the following two examples

Example 1 Hemorrhage from duodenal ulcer seven days before Red cells 2 2 millions Hb 5 7 Gm reticulocytes 6 per cent All three resistance distributions bimodal Saponin distribution has modes at 10γ and 43γ digitonin distribution has modes at 9 5γ and 12γ hypotonic NaCl distribution has modes at 0 520 and 0 360 tonicity units (fig 3)

Example 2 Hemolytic anemia accompanying metastatic carcinoma with pylorus as the primary site Red cells 1 8 millions Hb 4 6 Gm reticulocytes 13 per cent All distributions bimodal Saponin modes 10γ and 30γ digitonin modes 7 5γ and 9 4γ hypotonic NaCl modes 0 488 and 0 416 tonicity units (fig 4)

In these cases the bimodalities are presumably due to a new population with its own frequency distribution of resistances having been added to the existing one at the time of the hemorrhagic or major hemolytic episode The existing data do not allow the situation to be analyzed further It would be almost certainly wrong for example to identify one of the modes as being produced by the reticulocytes in the population All that can be said is that *at least* two distinguishable frequency distributions are superimposed

Illustrations of persistent abnormalities in the form of one or more of the frequency distributions are less striking and not so easy to supply Apart from the marked change in the distribution to saponin found in pernicious anemia in relapse[14] and to lysolecithin found in congenital hemolytic icterus [9] the abnormalities are usually encountered during routine determinations of the resistance distributions in hypoplastic and toxic anemias and in the anemias associated with leukemia the various forms of lymphoma etc The italicized values found in the following three cases are outside the normal range of variation as given in Table 1

THE HEMOGLOBIN OF HEALTHY COLLEGE UNDERGRADUATES AND COMPARISONS WITH VARIOUS MEDICAL SOCIAL PHYSIOLOGIC AND OTHER FACTORS

By Clark W Heath M D

AN UNUSUAL opportunity for obtaining data concerning the blood of healthy young college men has been afforded in the work of the Grant Study and the Harvard Fatigue Laboratory The subjects were Harvard undergraduates selected for good health and adjustments * In addition to medical examination and blood examination these men were studied by a variety of technics including physiologic tests anthropologic measurements and psychologic and social surveys The average age was 19 years 7 months the range 17 years to 25 years Sixty-one per cent were born in Northeastern United States The remainder were born elsewhere in the United States with the exception of three foreign born Table 1 indicates the distribution of hemoglobin findings and red blood cell counts in 153-59 of these young men

Methods

Sahli hemoglobin determinations and red blood cell counts were obtained on oxalated venous blood in the course of the physical examination Sahli tubes and solid glass standard were calibrated from bloods whose oxygen capacity had been ascertained converting to grams of hemoglobin by the factor 0 746 Oxygen capacities were determined by the Van Slyke method in the Fatigue Laboratory on heparinized arterial blood taken as a rule in the fasting state The two determinations were made at varying times apart usually a few days or weeks

The means of hemoglobins are only slightly less as a rule than those reported elsewhere in this country but higher than those reported from England † The wide variation of hemoglobins is worthy of comment In the total series of Grant Study participants only one case was omitted in which rather low hemoglobin seemed possibly the effect of a chronic infection In other instances of lower

From the Grant Study Department of Hygiene the Fatigue Laboratory Harvard University the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard) Boston City Hospital and the Department of Medicine Harvard Medical School

* Detailed description and method of selection of the men are given in reference 1

† M M Wintrobe[2] 86 male medical students Newcomer Method Mean 16 0 S D 0 9

MacFarlane and O Brien[3] 29 medical students and members of hospital staff Oxygen Capacity Method Mean 15 9

E E Osgood 137 medical students Osgood Method Mean 15 76 S.D 1.09

Andresen and Mugrage[5] 36 men of various ages Van Slyke Method Mean 16 58 S.D 0 8_

C Price Jones 00 men (London) of various ages Haldane Method Mean 14 55 S.D 0 54

C Price Jones Vaughan and Goddard[7] 90 men various ages Haldane Method Mean 14 55 S.D 0 69

R L Haden[8] 20 men ages 18 to 30 Van Slyke Method Mean 15 83 S D 0 76

P C Foster and Johnson 115 male students Van Slyke Method Mean 15 63 S.D 0 9

C J Hamre and Au[9] 137 university students (Honolulu) (varying racial groups) Acid Hematin and Van Slyke Methods Mean 15 10 S D 1 11

C F Nelson and Stoker[11] 350 men of various ages Van Slyke Method Mean 15.03 S.D 1 15

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and that are compatible with good function of their particular physiology It is hardly necessary to detail the wide variation of other measurements in any population such as height weight basal metabolism and pulse rate variations not necessarily due to health nutrition or other environmental influences

An attempt has been made to search for factors which might correlate with higher or lower hemoglobins in these 258 men selecting from the extensive data available in the Grant Study As will be seen the search was largely unavailing Only in the case of sitting pulse rate taken at the time of medical examination and blood collection was there a statistically significant relationship with hemoglobin level Other factors showed certain trends as will be indicated

The method of relating hemoglobin level to various factors is illustrated by table 2 The Chi-square (X) test [1] which compares observed results with those expected by chance was employed Table 2 shows that there is an excess of individuals having lower hemoglobins who have slower pulse rates and a deficiency of individuals with lower hemoglobins having faster pulse rates (The average

TABLE 2 —*Comparison of Hemoglobin and Pulse Rate (Sitting at time of Medical Examination)*

Pulse Rate	Hemoglobin					
	15.1 Gm 100 cc. or less		15 2 Gm./100 cc or more		Total	
	N	%	N	%	N	%
75 Beats per min or less		65 8	64	50 4	141	57 8
76 Beats per min or more	40	34 2	63	49 6	103	42 2
Totals	11	100 0	127	100 0	244	100 0

hemoglobin was 15 09 Gm for those having pulse rates of 75 or less 15 39 Gm for those having pulse rates of 76 or more) The Chi-square test applied to this four fold table shows that a relationship of this extent could occur by chance less than once in 50 times (P = 02) Arbitrarily this is taken as a significant relationship Comparison of Sahli hemoglobin with the hemoglobin by the Van Slyke method with red blood cell count with hematocrit shows much closer relationship than this as would be expected Hemoglobin level compared to each of the various factors that follow however never reached the probability of one in 50 (P = 02 and rarely one in 20 (P = 05)

Pulse rates during the physical examination determined in recumbent and standing positions also showed a tendency for the slower rates to accompany lower hemoglobin faster rates higher hemoglobin but not to the extent of sitting pulse rate and not significant by the described criterion Such tendencies however help to confirm the general observation that hemoglobin is often higher in individuals with faster pulse rates Since the pulse rate is a somewhat sensitive indicator of emotional state it is likely that the hemoglobin in these men has also reflected to some extent emotional disturbance or excitement toward the examination and the drawing of blood and although excitement was not a very marked response in these men it was quite obvious in some cases Increase of the red blood cells and

hemoglobin levels although determinations were repeated only the first determination is reported here Cases in which the hemoglobin level was at the extremes, as 13 5 grams or less and 16 0 grams or more gave no clinical evidence to explain such levels In one young man the initial Sahli hemoglobin was 13 1 Gm one week later 13 4 Gm six weeks after that 14 35 Gm finally, four days after the third determination his hemoglobin was 14 6 by the Van Slyke method Another subject was observed to have a hemoglobin level of 16 1 Gm Two years previously determinations at Thorndike Memorial Laboratory were 15 8 Gm and 17 2 Gm two weeks apart One month after the Sahli determination at the Grant

TABLE 1 —*Distribution of Hemoglobin Determinations and Red Blood Cell Counts*

Class Interval	Hemoglobin by Sahli Test		Hemoglobin by O Capacity		Class Interval R B C	Red Blood Cells	
	No Cases	%	No Cases	%		No Cases	%
Gm Hgb					*mils /c mm*		
12 6–12 9	1	0 4	1	0 7	4 20–4 29	1	0 4
13 0–13 3	1	0 4	1	0 7	4 30–4 39	3	1 2
13 4–13 7	8	3 1	8	5 2	4 40–4 49	1	0 4
13 8–14 1	18	7 0	10	6 5	4 50–4 59	1	0 4
14 2–14 5	36	13 9	26	17 0	4 60–4 69	8	3 1
14 6–14 9	27	10 5	25	16 3	4 70–4 79	16	6 2
15 0–15 3	53	20 1	25	16 3	4 80–4 89	24	9 3
15 4–15 7	43	16 7	26	17 0	4 90–4 99	37	14 3
15 8–16 1	50	19 4	16	10 5	5 00–5 09	71	27 4
16 2–16 5	19	7 4	9	5 9	5 10 5 19	50	19 3
16 6 16 9	2	0 8	2	1 3	5 20 5 29	31	12 0
17 0–17 3	0	0 0	3	2 0	5 30–5 39	11	4 2
17 4–17 7	0	0 0	0	0 0	5 40–5 49	4	1 5
17 8–18 1	0	0 0	1	0 7	5 50–5 59	1	0 4
Totals	258	99 7	153	100 1		259	100 1
Range	12 6–16 8 Gm		12 6–17 8 Gm			4 25 5 56 Mils	
Mean	15 19 Gm		15 13 Gm			4 98 Mils	
St dev	75 Gm		86 Gm			13 Mils	
Coeff var	4 9%		5 6%			2 6%	

Study the hemoglobin by the Van Slyke method was only 14 6 Gm With due consideration for the variations that may take place in the hemoglobin level of an individual from day to day as well as the possible errors in determination * it appears that individual persons have hemoglobin ranges that are native to them

* Even under carefully standardized conditions the errors in performing the Sahli test *upon the* same blood sample may be considerable In one series of observations when the test was repeated by the same observer only 13 per cent of the readings were identical 7 per cent were within 2 per cent or 0 31 Gm 95 per cent within 3 per cent or 0 4 Gm When the test was repeated by different individuals 19 per cent of the readings were identical only 32 per cent were within 2 per cent or 0 31 Gm only 44 per cent within 3 per cent or 0 47 Gm (Figure furnished by G A D land Thorndike Memorial Laboratory)

TABLE 5

	No. subjects
Socio-economic factors	
Location of birth compared to Sahli hemoglobin	250
Order of birth compared to Sahli hemoglobin	256
Family income compared to Sahli hemoglobin	255
Dietary† and gastro-intestinal factors	
Daily calories compared to Sahli hemoglobin	253
Daily protein in food compared to Sahli hemoglobin	253
Daily meat in food compared to Sahli hemoglobin	253
Daily fat in food compared to Sahli hemoglobin	253
Per cent fat in food compared to Sahli hemoglobin	253
Daily carbohydrate in food compared to Sahli hemoglobin	253
Daily iron in food compared to Sahli hemoglobin	253
Daily calcium in food compared to Sahli hemoglobin	253
Daily phosphorus in food compared to Sahli hemoglobin	253
Daily glasses of milk compared to Sahli hemoglobin	253
Daily Vitamin C in food compared to Sahli hemoglobin	253
Daily Vitamin D in food compared to Sahli hemoglobin	253
Estimate of appetite compared to Sahli hemoglobin	235
Daily candy consumption compared to Sahli hemoglobin	245
Speed of eating compared to Sahli hemoglobin	182
Effect of stress on G.I. function compared to Sahli hemoglobin	250
Hemorrhoidal tabs compared to Sahli hemoglobin	254
Frequency of bowel movements compared to Sahli hemoglobin	252
Regularity of bowel movements compared to Sahli hemoglobin	242
Physiologic and other factors	
Duration of treadmill run compared to Sahli hemoglobin	176
Lactic acid after run compared to Sahli hemoglobin	84
Recovery Index (treadmill) compared to Sahli hemoglobin	171
Basal metabolism compared to Sahli hemoglobin	171
Basal metabolism compared to O_2 Capacity	68
Mouth temperature compared to Sahli hemoglobin	250
Respiratory rate (medical exam.) compared to Sahli hemoglobin	258
Respiratory rate (basal) compared to Sahli hemoglobin	202
Tidal air compared to Sahli hemoglobin	206
Blood groups compared to Sahli hemoglobin	200
Blood groups compared to O_2 Capacity	114
Frequency of past illnesses compared to Sahli hemoglobin	254
Number of dental fillings compared to Sahli hemoglobin	231
Dental occlusion compared to Sahli hemoglobin	243
Dental eruptions (maturation) compared to Sahli hemoglobin	245
Integration of personality compared to Sahli hemoglobin	186
Bland versus vital personalities compared to Sahli hemoglobin	97
Mood swings trait compared to Sahli hemoglobin	259
Soundness of personality compared to Sahli hemoglobin	245
Date of blood examination compared to Sahli hemoglobin	257

(*Continued on next page*)

hemoglobin in excitement in man and animals has been described the so-called emotional polycythemia [13] A group of the present subjects who have been judged by the psychiatrist to have greater than average instability of the autonomic

TABLE 3

Pulse rate	No subjects
Basal compared to Sahli hemoglobin	178
Sitting before treadmill run * compared to Sahli hemoglobin	125
Standing before treadmill run compared to Sahli hemoglobin	126
At start of treatmill run * compared to Sahli hemoglobin	165
Maximum during treadmill run * compared to Sahli hemoglobin	172
One minute after treadmill run compared to Sahli hemoglobin	171
Two minutes after treadmill run * compared to Sahli hemoglobin	171
Four minutes after treadmill run * compared to Sahli hemoglobin	172
Sitting compared to O_2 Capacity	140
Recumbent compared to O_2 Capacity	80
Basal compared to O_2 Capacity	117
Sitting compared to red blood cells	245
Recumbent compared to red blood cells	253
Standing compared to red blood cells	188
Sitting compared to hematocrit	244
Recumbent compared to hematocrit	251

Treadmill run refers to a standardized work experiment carried out in the Harvard Fatigue Laboratory a description of which may be found in reference 14

TABLE 4

	No subjects
Systolic B P recumbent compared to Sahli hemoglobin	257
Systolic B P recumbent compared to O_2 Capacity	84
Systolic B P sitting compared to Sahli hemoglobin	245
Systolic B P standing compared to Sahli hemoglobin	187
Diastolic B P recumbent compared to Sahli hemoglobin	257
Diastolic B P sitting compared to Sahli hemoglobin	243
Diastolic B P standing compared to Sahli hemoglobin	197
Pulse Pressure recumbent compared to Sahli hemoglobin	256
Systolic B P one minute after treadmill run compared to Sahli hemoglobin	66
Systolic B P sitting compared to red blood cells	246

nervous system functions showed a tendency to have higher hemoglobins (as well as higher pulse rates) than those not so judged On the other hand no significant relationship was seen between hemoglobin level and a medical estimate of general reaction to stress or of immediate reaction to venipuncture

The additional comparisons shown in table 3 were made in the attempt to throw further light on the question of the relationships between the blood and pulse rate or emotional excitement

Comment

Such negative findings in a relatively homogeneous group of young men should not be unexpected They do not necessarily mean that relationships of hemoglobin with various environmental factors such as nutrition and disease incidence would not be present in larger population studies Certainly there have been shown to be distinct ranges of hemoglobin at different ages in the two sexes and in certain geographic areas where there are different nutritional influences The relatively large range of hemoglobins in healthy young men remains unexplained by the present study Emotional factors may be a partial explanation but future study will have to show the relative place of this and other factors

Summary

1 The distribution of hemoglobins (obtained by the Sahli method on venous blood and by the O capacity method of Van Slyke) and the red blood cell counts of 153 to 259 college men selected for participation in the Grant Study are reported

2 There is a statistically significant relationship between the hemoglobin and the pulse rate (those subjects having higher pulse rates tending to have higher hemoglobins) This relationship is apparently associated with emotional factors

3 No significant relationship could be established between hemoglobin level and a variety of other factors including socio-economic dietary physiologic medical body build and others

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TABLE 5—*Continued*

	No subjects
Body build factors	
Height compared to Sahli hemoglobin	253
Weight compared to Sahli hemoglobin	253
Height/√weight compared to Sahli hemoglobin	252
Surface Area compared to Sahli hemoglobin	253
Head circumference compared to Sahli hemoglobin	252
Chest circumference compared to Sahli hemoglobin	252
Head circ /Chest circ compared to Sahli hemoglobin	252
Umbilical circ /Chest circ compared to Sahli hemoglobin	204
‡Pyknic component compared to Sahli hemoglobin	253
Somatic component compared to Sahli hemoglobin	253
Leptic component compared to Sahli hemoglobin	253
Predominance of somatotype compared to Sahli hemoglobin	1 9
‡Masculine component compared to Sahli hemoglobin	253
‡Masculine component compared to O Capacity	152
Masculine component compared to hematocrit	252
*Masculine component compared to red blood cells	254
*Posture compared to Sahli hemoglobin	213

* Order of birth was divided into (1) only child or first born (2) second born (3) third born or later in rank of birth

† Dietary information was obtained from diet history of servings consumed and weighed average servings in the dormitory (See reference 1 page 128)

‡ There was a tendency not significant for the more pyknic (i e greater fatness and roundness and the less masculine body builds to have somewhat higher blood levels

None of these comparisons showed a significant relationship according to the Chi square test The *tendency* was however for faster pulse rates to be associated *with higher blood levels in all instances but the two when recumbent and standing* pulse rates were compared to red blood cell count The consistency of these trends confirms the impression that higher blood levels tend to be found among individuals with faster pulse rates It is possible of course that under the conditions of these observations a type of emotional response in some individuals may result in lower blood levels with elevated pulse rates It is worth noting that the trends became less significant as the effect of exercise on the pulse came into evidence No relationship between work performance and hemoglobin could be established

Comparisons with blood pressure were made as shown in table 4 Although no significance could be attached to the findings the trends were consistent enough to suggest the predominance of lower hemoglobin levels in individuals having higher systolic blood pressures and higher hemoglobin levels in those with higher diastolic pressures

The comparisons given in table 5 were made in order to explore the possible relationships between socio-economic dietary physiologic medical and body build factors with blood levels None of the comparisons showed significant relationships and no conclusions can be drawn other than that the hemoglobin level seems to be independent of these factors in healthy college men

TABLE 1 — The maximal and minimal findings and the average values of blood counts made on 40 males and 29 females at different times of the year

	January		March		June		October	
Average number for 40 males								
Hemoglobin (per cent)	100	(112 - 90)	97	(106 - 87)	99	(110 - 97)	99	(99 - 78)
Erythrocytes (millions)	5 06	(5 96 - 4 57)	4 94	(5 50 - 4 41)	5 00	(5 58 - 4 50)	4 96	(5 56 - 4 28)
Color index	0 99	(1 0 - 0 88)	0 98	(1 07 - 0 87)	0 99	(1 08 - 0 91)	0 90	(0 9 - 0 75)
Reticulocytes	4 2	(15 - 1)	4 2	(10 - 1)	5 8	(18 - 1)	4 6	(10 - 1)
Sedimentation rate	2 6	(6 - 1)	2 5	(10 - 1)	2 2	(6 - 1)	3 0	(5 - 1)
White cells	5830	(9440 - 3500)	5 00	(117 000 - 3320)	6050	(12 000 - 3400)	5900	(9080 - 3490)
Neutrophil	3150	(6600 - 1520)	3160	(6510 - 1300)	3590	(5 60 - 1400)	3280	(6950 - 1090)
Eosinophils	215	(480 - 72)	194	(450 - 90)	204	(510 - 51)	235	(490 - 60)
Lymphocytes	1900	(2610 - 995)	1800	(2510 - 1100)	1690	(2600 - 980)	1790	(2600 - 1260)
Monocytes	550	(9 0 - 240)	520	(1010 - 120)	530	(25 - 360)	560	(690 - 250)
Average numbers for 29 females								
Hemoglobin (per cent)	86	(100 - 70)	94	(97 - 95)	84	(95 - 74)	77	(89 - 72)
Erythrocytes (millions)	4 41	(5 10 - 3 6)	4 40	(5 30 - 3 82)	4 32	(5 01 - 3 75)	4 24	(4 75 - 3 78)
Color index	0 97	(1 0 - 0 79)	0 95	(1 0 - 0 84)	0 9	(1 06 - 0 90)	0 91	(0 99 - 0 80)
Reticulocytes	4 5	(13 - 1)	3 4	(10 - 1)	5 0	(11 - 1)	4 7	(10 - 1)
Sedimentation rate	5 2	(11 - 2)	4 5	(9 - 2)	3 6	(10 - 1)	5 5	(10 - 3)
White cells	5620	(8120 - 3640)	5430	(7440 - 3510)	5380	(7600 - 3940)	5570	(7480 - 4140)
Neutrophils	3210	(6100 - 1530)	3170	(5580 - 1690)	3220	(5410 - 1920)	3130	(4530 - 2050)
Eosinophils	162	(300 - 30)	1 6	(422 - 61)	179	(389 - 53)	169	(360 - 50)
Lymphocytes	1740	(2,70 - 1040)	1580	(2680 - 1100)	1490	(2090 - 1140)	1740	(2430 - 1120)
Monocytes	510	(60 - 250)	490	(880 - 270)	450	(920 - 240)	510	(1070 - 250)

NORMAL BLOOD COUNTS IN DIFFERENT SEASONS

By J Engelbreth Holm M D and Aa Videbaek M D

BLOOD counts like various other routine examinations were introduced into clinical medicine a rather long time before their physiologic variations and the ranges of their normal values had been finally determined Hence all statements of leukocyte counts and differential counts in normal individuals suffer from want of uniformity Sex and age and perhaps even race must be considered Exercise and excitement bring about a conspicuous increase of the number of leukocytes whereas variations due to static changes are less significant [4] The alleged digestive leukocytosis has not been confirmed by modern investigations [9] Further diurnal rhythms of the neutrophils have been noticed [6] presenting a climax in the afternoon and shortly after midnight Likewise rhythmical variations have been observed showing maxima and minima within about one hour [6] According to Friedlander and Wiedemer [3] the number of reticulocytes rises conspicuously during the months of spring dropping to a low in the autumn whereas inversely the hemoglobin and the number of erythrocytes show the lowest values during spring Further these authors have stated that heliotherapy is followed by a rise in the number of reticulocytes they therefore suggest that seasonal variations in the reticulocyte count must be due to changes in the intensity of sunlight

In order to decide whether results of routine blood examinations are subject to seasonal variations worth mentioning we have carried out examinations four times during one year (in the months January March June and October) on 40 male and 29 female healthy students of medicine determining the hemoglobin per cent (Haldane) the number of erythrocytes and the number of reticulocytes (per one thousand erythrocytes) and the sedimentation rate counting in addition white cells and eosinophils (Dunger) and making differential counts of 300 cells on cover glass smears The examinations were performed in the morning on venous blood after half an hour of muscular rest all the tests being examined by the same person

The hemoglobin value for both sexes drops to its lowest in October the difference being statistically significant reduction of the average figures is about 10 per cent equally the color index seems to be lowest in October the number of erythrocytes however being constant The highest reticulocyte numbers are found for both sexes in the most sunny periods of the year the deviation being however not significant whereas the sedimentation rate is found to be lowest in June and highest in October for both sexes The white cell count and that of their fractions present great variations although the averages vary but slightly and insignificantly it must however be kept in mind that the choice of the four months of examination has been arbitrary and contingent maxima might fall outside these periods

The results present a conspicuous variation especially from one *individual to* another at a given time of the year whereas variations in the same person examined

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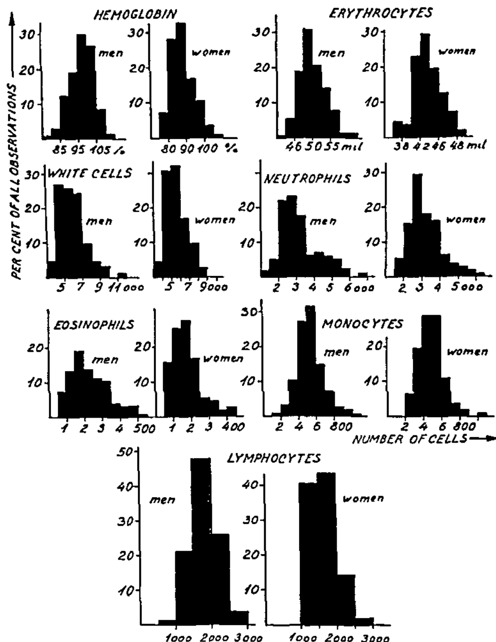


FIG 1 SHOWING BLOOD COUNTS FOR 40 MEN AND 29 WOMEN EXAMINED FOUR TIMES DURING ONE YEAR

The diagrams show the percentage of all observations occurring within the different intervals

SUMMARY

Report is given of examinations carried out on 40 healthy males and 29 healthy females in the months of January March June and October with determination of

at different seasons are less pronounced This indicates that physiologically some individuals have a high, and others a low leukocyte level The averages for the 40 males and 29 females examined at different seasons will be seen in table 1 The seasonal variations which have been observed are less pronounced than the individual variation at a fixed time of the year in other words the seasonal variation is without significance to the estimation of the results achieved by common blood counts *The present figures thus yield a basis of evaluaton of the normal average* values for adult men and women The results may be seen from table 2 showing further the maximal and minimal findings the marked standard deviation is illustrated in figure 1 Since each leukocyte group may present relatively high per cent values even with a low leukocyte total and vice versa there can be ascribed no special importance to the cell percentage which must be considered only as an aid to

TABLE 2 —*The maximal and minimal findings and the average values of blood counts made on 40 males and 49 females at different times of the yea*

	Men		Women	
Hemoglobin (per cent)	96	(78 – 112)	82	(70 – 100)
Erythrocytes (millions)	4 99	(4 28– 5 96)	4 35	(3 75– 5 30)
Color index	0 97	(0 75– 1 08)	0 95	(0 79– 1 07)
Reticulocytes	4 7	(1 – 10)	4 4	(1 – 13)
Sedimentation rate	2 6	(1 – 10)	4 6	(1 – 11)
White cells	5870	(3320 –12 000)	5500	(3520 –8120)
Neutrophils	3310	(1090 – 6850)	3190	(1530 –5820)
Eosinophils	210	(51 – 510)	170	(30 – 422)
Monocytes	540	(117 – 1014)	490	(240 –1070)
Lymphocytes	1800	(980 – 2610)	1640	(1040 –2770)
Neutrophils (per cent)	55 0	(38 7 – 74 5)	57 4	(42 4 – 75 7)
Eosinophils (per cent)	3 4	(0 – 10 0)	2 9	(0 5 – 8 0)
Monocytes (per cent)	9 4	(4 0 – 14 0)	8 9	(5 3 – 14 3)
Lymphocytes (per cent)	31 6	(11 3 – 47 0)	30 0	(15 0 – 45 0)

the estimation of the absolute number of the different white blood cells A lymphocyte per cent of about 50 thus may very well be normal but this is conclusive only when the absolute number is to be found within the ranges of 1000–3000 The lines drawn by Naegeli and Schilling [5, 8] 20–25 per cent and 21–35 per cent respectively therefore must be considered too narrow whereas the absolute figures come up very well to the statements given by e g Boerner [1] Schilling [8] and Saltzmann [7] It is however worth mentioning that the upper normal range of monocytes and eosinophils is 1000 and 500 respectively According to the above examinations the normal values for adult men and women are

White cells	3 000–10 000 per cu mm of bl d
Neutrophils	1 000 6 000
Eosinophils	50–500
Monocytes	100 1 000
Lymphocytes	000 3 000

THE OCCURRENCE OF NORMOBLASTS IN THE PERIPHERAL BLOOD IN CONGESTIVE HEART FAILURE AN INDICATION OF UNFAVORABLE PROGNOSIS

By J Groen M D and E G Godfried M D

DURING THE PAST year the authors have observed 9 patients with severe congestive heart failure all of whom showed a varying number of normoblasts in the peripheral blood In some of these cases there was a temporary remission of the sequellae of the heart failure during this remission the normoblasts disappeared from the peripheral blood However in all cases the patients died

Case Reports

Case 1 C D a 34 year old woman was admitted on June 12, 1946 because of decompensated mitral stenosis She was slightly jaundiced the liver was greatly enlarged there were infarcts in both lungs Five per cent normoblasts were found in the peripheral blood The patient died on July 2 1946 The diagnosis was confirmed at autopsy

Case 2 S W a 56 year old woman entered the hospital on May 9 1946 because of dyspnea from which she had been suffering during the previous six months She was cyanotic dyspneic and had slight jaundice On examination a mitral insufficiency stenosis and auricular fibrillation were found The liver was much enlarged there was extensive edema During this period 10 and 17 per cent respectively of normoblasts were found on two occasions in the peripheral blood but these disappeared within three days when her condition improved In spite of this remission the patient died on May 22 1946 On the day before her death she had another infarction of the lung

On May 13 a sternal puncture had been performed The bone marrow was found to be essentially normal but the presence of a relatively great number of normoblasts and erythroblasts was noted (about 25 per cent of all nucleated cells)

The diagnosis of valvular heart disease was confirmed at autopsy there were many infarcts in both lungs and a thrombosis of the left auricle of the heart

Case 3 A G a 20 year old woman entered the hospital on May 4 1946 with cardiac decompensation due to insufficiency and stenosis of the mitral valve She was dyspneic but not cyanotic There was no jaundice The liver was enlarged There was massive edema of the leg The blood count was as follows

	May 6 1946	May 7 1946
Leukocytes	12 800	17 000
Erythrocytes	3 640 000	3 900 000
Hemoglobin	61	63
Normoblasts	—	7
Myelocytes	1	0 5
Metamyelocytes	0 5	0 5
Stabs	5 5	9
Polynuclear cells	54 5	69
Eosinophiles	0 5	—
Monocytes	2 5	2 5
Lymphocytes	35 5	18 5

There was a marked anisocytosis poikilocytosis polychromasia anisochromia and macrocytosis The patient died on May 8 1946 The autopsy showed a verrucous endocarditis (of the mitral and aortic valves) thrombi in the left auricle of the heart congestion fatty degeneration and regeneration of the liver

Case 4 A B a 34 year old man was admitted on June 28 1946 with a diagnosis of insufficiency and

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the hemoglobin the number of erythrocytes and reticulocytes the sedimentation rate the white cell and differential counts The hemoglobin value appears to be lowest in October the number of reticulocytes highest in June the sedimentation rate lowest in June The remaining figures present no exact seasonal variations Some individuals have a high leukocyte level others a low one Normal values are illustrated

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in addition she had a tricuspid stenosis and there was a thrombus in the right atrium The liver was cirrhotic

Case 4 J D a 63 year old woman was admitted on November 13 1946 with a diagnosis of mitral insufficiency and stenosis with auricular fibrillation and decompensation She was slightly dyspneic and deeply cyanotic There was no jaundice Infarctions were present in both lungs The blood count was as follows leukocytes 5 600 erythrocytes 4 300 000 hemoglobin 88 normoblasts 2 polynuclear cells 66 eosinophiles 2 monocytes 5 lymphocytes 27

The patient died on February 5 1947 The clinical diagnosis was confirmed at autopsy there were multiple infarctions in both lungs

Discussion

The appearance of normoblasts in the blood of patients with congestive heart failure is mentioned only twice in the literature The standard textbooks of diseases of the blood and of the heart describe the finding of normoblasts in the peripheral blood only in relation to congenital heart disease accompanied by cyanosis In

TABLE 2a—*Cabot's cases*

Patient	Age	Clinical data	Hematologic data	Remarks
Case I	8	Mitral disease nephritis	Hb 35 2% normoblasts leukocytes 220 000	Died
Case II	25	Mitral disease aortic insufficiency	Hg 35 1% normoblasts leukocytes 13 000	Died
Case III	30	Mitral disease aortic insufficiency	1st day Hb 10 8% normoblasts leukocytes 6 000 7th day Hb 10 4% megaloblasts % normoblasts leukocytes 4 200 15th day Hb 15 leukocytes 10 400 20th day Hb 12 5 2% megaloblasts leukocytes 5 000	?

acquired valvular disease and in other forms of congestive heart failure this sign seems to have been overlooked by the majority of investigators

Cabot[1] mentions in the fifth edition of this book The Clinical Examination of the Blood three cases with decompensated mitral disease in which a severe anemia was present The blood contained nucleated red cells All three were chronic cases without active endocarditis He does not give further details and there are no data regarding the presence of infarcts in the lungs Two of Cabot s patients died the fate of the third is not mentioned

In 1931 Frank and Hartmann described six patients with various types of right sided heart failure in whom normoblasts were found in the peripheral blood All cases showed cyanosis and some degree of jaundice and all of them died The cases of Frank and Hartmann were not anemic

Table 2 summarizes the authors observations and some of the data on the cases described by Cabot and Frank and Hartmann From this table it is evident that all the cases showed a very severe insufficiency of the heart It is also clear that it was

stenosis of the mitral valve and aortic stenosis He was cyanotic and extremely dyspneic There was slight jaundice The liver was palpable There was no edema The patient had distinct anginal pain An electrocardiogram showed changes that were typical of a severe myocardial lesion The blood picture was as follows leukocytes 7 000 erythrocytes 5 000 000 hemoglobin 100 normoblasts 3 stabs 4 polynuclear cells 80 monocytes 1 lymphocytes 15

The patient died on July 5 1946 No autopsy was performed

Case 5 H S a 71 year old man was admitted on March 4 1946 with cardiac asthma He had been known for years to have suffered from hypertension The patient was extremely cyanotic There was slight jaundice and very severe dyspnea He showed all the signs of congestive heart failure There was extensive edema of the legs ascites and hydrothorax One normoblast was found per 100 nucleated cells When the signs of heart failure disappeared normoblasts were no longer seen The patient died November 4 1946 Autopsy showed hypertrophy of the heart multiple emboli in both lungs and chronic glomerulonephritis

Case 6 P H a 48 year old woman was admitted on November 9 1946 with mitral insufficiency and stenosis She was dyspneic and cyanotic No jaundice was present There was gangrene of the tip of the nose and of both lower legs No pulsations were felt in the left femoral and the right popliteal arteries There was an infarct in the right lung The blood count was as follows leukocytes 22 000 erythrocytes

TABLE 1 —*Blood Picture of S W (Case 2)*

	5/10	5/11	5/13	5/14	5/16	5/18	5/21
Leukocyte count	12 500			12 700	12 400		26 000
Erythrocyte count	5 250 000				5 360 000		5 310 000
Hemoglobin	77				80		82
Normoblasts	10	17	3				
Myelocytes			1				
Metamyelocytes	1	1					
Stabs	4	7	4	4	4	4	7
Polynuclear cells	82	81	86	88	89	79	84
Monocytes	1	2	1	4	3	1	
Lymphocytes	12	9	8	4	4	16	9

4 020 000 hemoglobin 59 normoblasts 1 stabs 6 polynuclear cells 80 eosinophiles 1 monocytes 3 lymphocytes 10

The patient died on November 16 1946 The autopsy revealed a thrombus in the left auricle of the heart an infarct of the lower lobe of the right lung an embolic obturation of the left iliac artery and congestion of the liver

Case [illegible] T V a 45 year old woman was admitted on November 19 1946 She had a history of chorea twenty seven years previously A diagnosis of mitral insufficiency and stenosis was made The patient had auricular fibrillation and multiple infarctions of both lungs There was gangrene of the tip of the nose and of the right foot the latter was caused by an obturation of the right femoral artery The patient was extremely dyspneic and deeply cyanotic There was no jaundice An electrocardiogram showed bundle branch block The blood count was as follows leukocytes 2 800 erythrocytes 4 500 000 hemoglobin [illegible]8 normoblasts 4 myelocytes 1 metamyelocytes 1 stabs 1 polynuclear cells 78 monocytes 1 lymphocytes 14

The patient died on November 20 1946 At autopsy the clinical diagnosis was confirmed valvular heart disease thrombosis of the right auricle multiple infarctions in both lungs A small amount of fluid was found in both pleural cavities Cardiac cirrhosis of the liver with regeneration was also present

Case 8 T S a 40 year old woman was admitted for the first time on April 24 1946 because of mitral insufficiency and stenosis with auricular fibrillation She had a greatly enlarged liver with ascites The patient left the hospital on July 6 and was readmitted on August 30 On this second admission he was extremely dyspneic cyanotic and icteric On October 1 a few normoblasts were found in the peripheral blood The patient died on October 14 1946 At autopsy the mitral valve lesions were found

TABLE 21 — Author's observations

Patient	Age	Clinical data	Hematologic data	Remarks
Case I	34	6/12 Stenosis and insufficiency of mitral valve auricular fibrillation congestion of lungs orthopnea cyanosis edema of legs 6/15 Jaundice 6/17 Infarction in lung 6/30 Greatly enlarged liver /2 Died	6/19 3% normoblasts 6/21 3% normoblasts Hb 59 6/25 1% normoblasts 6/2 1% normoblasts	Autopsy Chronic pancarditis hypertrophy of heart thrombosis in both ears of heart infarction in lung edema of legs
Case II	36	5/9 Insufficiency and stenosis of mitral valve auricular fibrillation enlarged liver edema of legs orthopnea cyanosis slight jaundice 5/13 Injection of salyrgan followed by a large diuresis 5/22 Died	5/10 Hb 7 10% normoblasts leukocytes 12 500 5/11 17% normoblasts 5/13 4 hrs after injection of salyrgan 3% normoblasts From 5/14 onward no normoblasts	5/12 Sternal puncture—bone marrow almost normal 25% of nucleated cells are normoblasts and erythroblasts Autopsy Recurrent endocarditis of mitral valve hypertrophy of heart infarction in lungs thrombosis of left heart ear
Case III	20	5/4 Insufficiency and stenosis of mitral valve enlarged liver edema of leg 5 8 Died	5 6 Hb 61 no normoblasts leukocytes 12 800 5/7 7% normoblasts leukocytes 17 000	Autopsy Verrucous endocarditis (mitral and aortic valves) thrombi in left heart ear congestion of the liver with fatty degeneration and regeneration
Case IV	34	6/28 Insufficiency and stenosis of mitral valve stenosis of aortic valves enlarged liver cyanosis extreme dyspnea slight jaundice no edema /5 Died	Hb 100 3% normoblasts leukocytes 7 000	No autopsy
Case V	1	3 4 Decompensated hypertension cardiac asthma extreme dyspnea and cyanosis slight jaundice hydrothorax ascites gangrene and edema of leg 3 10 Regression of decompensation 8 30 Many attacks of dyspnea general condition poor 11 4 Died	3 4 1% normoblasts leukocytes 1 300 3/10 no normoblasts	Autopsy Hypertrophy of heart emboli of lungs chronic nephritis

TABLE 2b —*Frank and Hartmann Cases*

Pat e t	Age	Clinical d t	Hematologic data	Rema ks
Case I	52	Anginal complaints dyspnea pleural transudate anasarca	Hb 92 7% erythroblasts	Autopsy Coronary sclerosis myodegeneration of heart aneurism of left ventricle with thrombosis
Case II	37	Insufficiency and stenosis of mitral and aortic valves insufficiency of tricuspid valve decompensation enlarged liver slight jaundice infarctions in lungs	Hb 48 1% macroblasts *4% normoblasts leuko*cytes 25 000	Died No autopsy
Case III	39	2 years before admission amputation of leg for gangrene caused by endarteritis obliterans pneumonia slight jaundice	Hb 90 4% erythroblasts leukocytes 17 200 3 days later Hb 88 14% erythroblasts leukocytes 19 000 5 days later Hb 87 per 100 white cells 175 erythroblasts 14 macroblasts leukocytes 14 800	Autopsy Thrombus in left ventricle and right atrium arteriosclerosis and formation of a thrombus in right coronary artery myocardial infarction Thrombangitis obliterans of abdominal aorta
Case IV	54	*Coronary thrombosis* edema of legs jaundice	*Hb 67 4% erythroblasts* leukocytes 10 000	*Autopsy Coronary* thrombosis aneurism of left ventricle of the heart with a large thrombus
Case V	34	Strong cyanosis edema	2nd day Hb 87 per 100 hite cells 64 erythroblasts 6 macroblasts leukocytes 21 400 3rd day Hb 87 per 100 white cells 52 macroblasts 25 erythroblasts leuk cytes 17 700	Autopsy Hypertrophy of right ventricle thrombi in left ventricle thrombosis of right pulmonary artery
Case VI	42	Insufficiency and stenosis of mitral al e insufficiency of tricuspid valve extreme yan sis extreme dy pnea ja n dice edema	5th day Hb 78 2% normoblasts leukocytes 10 500 11th day 1% macroblasts 2% erythroblasts 12th day % erythroblasts 3% macroblasts 13th day 4% erythroblasts	Autopsy Chronic endocarditis of mitral and *tricuspid val e atrophy* and regeneration of liver jaundice

the decompensation and not any specific type of heart disease that caused the normoblastosis Among the patients showing the phenomenon there were cases

TABLE 2c — Authors' observations

Patient	Age	Clinical data	Hematological data	Remarks
Case I	34	6/12 Stenosis and insufficiency of mitral valve auricular fibrillation congestion of lungs orthopnea cyanosis edema of legs 6/15 Jaundice 6/17 Infarction in lung 6/30 Greatly enlarged liver 7/2 Died	6/19 5% normoblasts 6/21 3% normoblasts Hb 59 6/25 1% normoblasts 6/2 1% normoblasts	Autopsy Chronic pancarditis hypertrophy of heart thrombosis in both ears of heart infarction in lung edema of legs
Case II	56	5/9 Insufficiency and stenosis of mitral valve auricular fibrillation enlarged liver edema of legs orthopnea cyanosis slight jaundice 5/13 Injection of salyrgan followed by a large diuresis 5/22 Died	5/10 Hb 77 10% normoblasts leukocytes 12 500 5/11 1 % normoblasts 5/13 4 hrs after injection of salyrgan 3% normoblasts From 5/14 onward no normoblasts	5/12 Sternal puncture—bone marrow almost normal 25% of nucleated cells are normoblasts and erythroblasts Autopsy Recurrent endocarditis of mitral valve hypertrophy of heart infarction in lungs thrombosis of left heart-ear
Case III	20	5/4 Insufficiency and stenosis of mitral valve enlarged liver edema of leg 5/8 Died	5/6 Hb 61 no normoblasts leukocytes 12 800 5/ 7% normoblasts leukocytes 1 000	Autopsy Verrucous endocarditis (mitral and aortic valves) thrombi in left heart-ear congestion of the liver with fatty degeneration and regeneration
Case IV	34	6 28 Insufficiency and stenosis of mitral valve stenosis of aortic valves enlarged liver cyanosis extreme dyspnea slight jaundice n edema 7/5 Died	Hb 100 3 % normoblasts leukocytes 7 000	No autopsy
Case V	71	3 4 Decompensated hypertension cardiac asthma extreme dyspnea and cyanosis slight jaundice hydrothorax ascites gangrene and edema of legs 3 10 Regression of decompensation 8/30 Many attacks of dyspnea general condition poor	3 4 1% normoblasts leukocytes 1 300 3 10 no normoblasts	Autopsy Hypertrophy of heart emboli of lungs chronic nephritis

TABLE 2c —*Continued*

Patient	Age	Clinical data	Hematologic data	Remarks
Case VI	48	11/9 Insufficiency and stenosis of mitral valve dyspnea and cyanosis gangrene of tip of nose and both legs below knee obturated left femoral artery and right popliteal artery infarction in lung 11/16 Died	11/9 Hb 59 1% normoblasts leukocytes 22 000	Autopsy Chronic endocarditis stenosis of mitral valve thrombus in left heart ear embolic obturation of left iliac artery infarction in lung congestion of liver
Case VII	45	11/19 Insufficiency and stenosis of mitral valve auricular fibrillation decompensation of heart infarction in lung gangrene of tip of nose and of right foot extreme dyspnea and extreme cyanosis bundle branch block 11/20 Died	11/19 Hb 78 4% normoblasts leukocytes – 800	Autopsy Chronic endocarditis stenosis of mitral valve thrombus in right auricle of heart cardiac cirrhosis of liver with regeneration infarctions in lungs
Case VIII	40	4/24 Insufficiency and stenosis of mitral valve auricular fibrillation cirrhosis of liver dyspnea cyanosis slight jaundice 10 14 Died	10/1 Hb 78 1% normoblasts leukocytes 10 600	Autopsy Chronic endocarditis of mitral aortic and tricuspid valves thrombus in right atrium cirrhosis of liver
Case IX	63	11/13 Insufficiency and stenosis of mitral valve auricular fibrillation decompensation of heart slight dyspnea extreme cyanosis infarction of lungs 1–/11 General condition poor 2/5 of next year Died	11/15 Hb 88 2% normoblasts leukocyte 5 600	Autopsy Chronic endocarditi of mitral valve infarctions in lungs

of decompensated valvular heart disease as well as cases of heart failure in hypertension or in coronary thrombosis In Cabot s cases it might be argued that the normoblastosis was a result of the anemia rather than of heart disease but in all other cases anemia played no role The predominant factor in these nonanemic cases seemed to be a marked diminution of the oxygenation of the blood in the lungs as evidenced during life by severe cyanosis After death this was explained

in many cases by the finding of a thrombus in one of the auricles or ventricles of the heart and/or infarcts of the lungs Three of Frank and Hartmann s cases had myo cardial infarction with secondary formation of a mural thrombus in the left ven tricle One patient had a thrombosis of the right pulmonary artery Two patients had severe decompensation as a result of mitral stenosis In all our cases thrombosis or embolism inside the heart or pulmonary arteries was found eight times this was verified at autopsy Apart from thrombi and emboli in the heart and lungs the postmortem examinations showed no changes which could have caused the normoblastosis Fatty degeneration of the liver was regularly present cirrhosis and regeneration of bile ducts were sometimes prominent features No extramedul lar blood formation was found

Taking into consideration Cabot s anemic cases the conclusion is justified that peripheral normoblastosis occurs especially in those patients with heart failure who

TABLE 3

No	Age	Diagnosis	Normoblasts	Duration of life after first detection of normoblasts	Cyanosis	Dyspnea	Thromb in heart	Infarcts in lung
			per cent					
1	34	Mitral disease	5	13 days	+	++	+	+
2	56	Mitral disease	10	12 days	+	+	+	+
3	20	Mitral disease	7	1 day	−	+	+	−
4	34	Mitral disease aortic stenosis	3	7 days	+	++	?	−
5	72	Decompensated hypertension	1	10 months	++	++	−	+
6	48	Mitral disease	1	7 days	+	+	+	+
7	45	Mitral disease	4	1 day	++	++	+	+
8	40	Mitral disease tricuspid ste nosis	1	13 days	+	+	+	−
9	63	Mitral disease	2	3 months	++	++	−	+

are either markedly cyanotic or strongly anemic so that it appears as if anoxia is the most important cause of the normoblastosis We are inclined therefore to regard the occurrence of normoblasts in the peripheral blood as an indication of an attempt on the part of the body to increase the number of circulating red cells as a result of the stimulus which anoxia exerts on the blood forming apparatus Apparently normoblasts appear in the peripheral blood only when anoxia is ex treme in a degree that occurs only in the very severe forms of heart failure

Decompensation alone does not seem to produce the phenomenon it requires the presence of thrombi and/or infarcts in the lesser circulation This is probably the reason why Walter Blumgart and Volk[3] did not find normoblasts in the blood in their cases of congestive heart failure as they excluded all cases with complica tions from their study They noted however that there was an increase in reticu locytes in the peripheral blood in heart failure which disappeared when the condi tion improved

The presence of normoblasts in the peripheral blood in heart failure thus seems to indicate that the condition is complicated by mural thrombosis in the heart or

pulmonary artery, or by pulmonary emboli or a combination of these conditions Hence it is easily understood why peripheral normoblastosis is a sign of such poor prognosis

In some but not in all cases the normoblastosis was accompanied by a leukocytosis and (or) the presence of young precursors of the myeloid group Some cases showed not only a peripheral normoblastosis but a distinct leuko-erythroblastic blood picture This was most pronounced in one of Frank and Hartmann s patients who had a total white count of 21,000 and not less than 70 per cent nucleated red cells

Summary

The authors recommend the search for normoblasts in the blood of patients with severe heart failure When normoblasts are found a marked interference with the oxygenation of the blood either by pulmonary infarcts or thrombi inside the heart is most likely to be present It seems justifiable to consider the prognosis as very grave in these cases This rule proved to hold even in those cases where concomitant with an improvement in the heart failure, the normoblasts disappeared temporarily from the peripheral blood

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INFECTIOUS MONONUCLEOSIS

By Sir Henry Tidy

E PFEIFFER, a pediatrician of Vienna gave in 1889 the first clear description of the disease which we are here discussing under the title Drusenfieber or glandular fever He recognized that it was infectious and occurred in epidemics described accurately the course of the enlargement of the cervical glands in young children though he denied that other glands were involved and stated that the glands never suppurated and that the prognosis was uniformly favorable

Other observers in Germany quickly reported epidemics Spread of its knowledge was somewhat slow but Park West (1896) reported an epidemic in America and Dawson Williams (1897) in England The disease appeared to be successfully launched but the diagnosis in sporadic cases rested on rather indefinite clinical features It soon became confused with septic infections and rapidly fell into disfavor By 1900 it was practically dead No mention of it appears in the Medical History of World War I although information subsequently collected proves that it was not uncommon It remained in suspended animation until 1920

It is remarkable that during a period of thirty years covering rapid developments in the knowledge of hematology there should have been no systematic examination of the blood in a condition characterized by enlargement of lymphatic glands and often by enlargement of the spleen To this lack of observation there is one exception J E Burns in 1908 fully described the lymphocytosis in two epidemics which he diagnosed as glandular fever and published a well-documented report The communication was completely overlooked and I was unaware of its existence until it was quoted by Bernstein (1940) in his monograph Priority for the recognition of infectious mononucleosis clearly belongs to Burns

Nevertheless the establishment of the hematologic features of the disease did not come for practical purposes until 1920 Sprunt and Evans in November 1920 published their observations on transient mononucleosis recorded in a series of six young adults during the previous six years They recognized that the condition was infectious observed the general glandular enlargement and gave an accurate though brief description of the various types of cells in the blood They were clearly unaware of the existence of Pfeiffer s glandular fever and thought they had discovered a new disease—a very venial mistake—and named it infectious mononucleosis

Authoritative articles soon followed Longcope in 1922 described fully the clinical aspects He noted the long febrile period which might occur and suggested the possibility of encephalitis in one case Downey and McKinlay in 1923 gave a complete description of the cells beautifully illustrated to which nothing effective has been subsequently added

Meanwhile Morley and I in June 1920 recognized the existence of a transient lymphocytosis in the case of a boy whom we diagnosed as suffering from glandular

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fever and we reported it with other evidence at a meeting of the Royal Society of Medicine in December 1920 At that time we were unaware of the article by Sprunt and Evans but our attention was called to it when our article was in type and we formed the opinion that it was the same entity In the course of the next few years I saw a number of epidemics in boys schools in England and also cases in adults

The recognition of the identity of infectious mononucleosis and glandular fever was not immediate in America though Longcope used both terms in 1922 but it was probably general by 1925 The material on which the observations were based in America was predominantly from college students about the ages of 18 to 24 years while in England it was supplied by resident preparatory school boys of 8 to 14 years Although any type of the disease may occur at any age there are considerable differences when numbers of individuals are involved between the clinical manifestations in the two age groups the glandular enlargement being more marked at the younger ages These factors no doubt account for the difference in nomenclature for the disease is invariably known as infectious mononucleosis in America while in Britain it is generally named glandular fever On the continent of Europe the disease in young boys is now frequently described as Pfeiffer s glandular fever and that in adults as infectious mononucleosis the identity of its infection in the two groups being accepted

Neither resident college life nor resident preparatory schools are a part of the educational system in Europe outside Britain and the recognition on the continent of a recoverable mononucleosis (other than that of whooping cough) was based on a different type of material garbed in entirely other guise from that presenting in America and England Deussing in 1918 reported a series of cases with transient absolute lymphocytosis under the title Uber diphtherieahnlicher Anginen mit lymphatischer Reaction (Angina resembling diphtheria with a lymphatic reaction) But it was a communication by Schultz in 1922 to the Congress for Internal Medicine on Monozytenangina which first attracted attention to the subject Both communications were based on the same type of material being in regard to patients admitted to a hospital for communicable diseases who had membranous tonsillitis in which no diphtheria bacilli were found and in whom recovery followed without the injection of antitoxin

The continental authorities went astray from the start They were obsessed with the idea that the development of the lymphocytosis was due to a constitutional peculiarity of the patient resulting in a lymphatic reaction and that the same angina in other individuals would result in a polynucleosis The possibility of infection was scarcely considered Secondly they embarked on a tedious and sterile dispute amongst themselves covering many years as to whether the cells were monocytes or lymphocytes They failed to recognize that both types of cells were often present at the same time and that either type might predominate at different periods in the same patient Not until Glanzmann s monograph in 1930 did they admit that monocytic angina was a manifestation of infectious mononucleosis and recognize the influence of an infective factor as opposed to a constitutional diathesis

The definite differentiation of monocytic angina from diphtheria was a clinical

advance of importance and it is somewhat surprising that so little notice was taken of it in American and British literature The identity of monocytic angina with infectious mononucleosis was accepted without discussion by the specialists on communicable diseases but its existence was certainly not generally known to the profession in Britain even to the commencement of World War II A brief clinical description of this type follows

Monocytic angina or the anginose type of infectious mononucleosis is characterized by the development of a tonsillar membrane or of ulceration The membrane in typical cases is indistinguishable in appearance from that of diphtheria although it is a true membrane and it often forms very rapidly Edema of the neck and tenderness of the enlarged cervical glands is common but suppuration is very rare Edema of the fauces causes great discomfort and anxiety but in spite of this and the high temperature the patient does not appear to be severely toxic nor does he become so although the membrane may persist for several days or more than a week before separating after which the symptoms improve with surprising rapidity There is a previous period of slight malaise for two or three weeks with increasing sore throat and some glandular enlargement may or may not have been observed

There is no proof that the disease is infectious at this stage or that it spreads in this form although a number of cases may occur in a unit of young adults It is possible that the angina is a complication connected with the leukopenia which is often present initially before the mononucleosis develops

An extensive epidemic in England in 1930 was characterized by the severity and long duration of the attacks and by the high proportion of adults involved During an initial febrile period which might last several weeks the constitutional symptoms in this type are often suggestive of typhoid and the characteristic features of infectious mononucleosis absent Glandular enlargement develops late and is rarely of any great extent The blood at the onset may show a definite polynucleosis In prolonged cases this may be observed to subside and for a period the blood count may be strictly within normal limits or passing to a leukopenia The mononucleosis tends to develop about the time of the glandular enlargement and with their appearance the constitutional symptoms improve often very rapidly and the patient becomes convalescent

Sporadic cases of this type are not uncommon and one instance was included in Longcope s article in 1922

Four groups of clinical manifestations have so far here been indicated (1) Pfeiffer s glandular fever in children characterized by rapid and visible swelling of the cervical glands and a short duration (2) infectious mononucleosis in young males with a longer but milder febrile stage and comparatively slight glandular swelling (3) monocytic angina and (4) long febrile types with late and slight enlargement of glands

This grouping was useful while the clinical features of the disease were being carefully studied but the disease is apparently a single entity and every permutation and combination of the four groups occur Milder cases often fall fairly clearly into one group but this is rarely so for the severer forms

The Blood Picture

It is probable that mononucleosis develops in every case of infectious mononucleosis

All the blood forming tissues are affected myeloid monocytic (or reticulo endothelial) and lymphoid but at different times and to different degrees and varying in different cases and indeed in the same case at different stages The effect on one system may be decreasing while on another it is increasing thus producing the rapid changes in the blood picture which is so characteristic of the disease The sequence of the changes is best observed in the long severe febrile cases but unfortunately the diagnosis is rarely made in the early stages

The myeloid system is earliest involved but less constantly or severely and for a shorter time than the other systems In mild cases there may be no change in the circulating myeloid cells but in severer forms an initial polynucleosis such as 15–20 000 leukocytes with 75 per cent polynuclears is not infrequent This initial polynucleosis is a common cause for the diagnosis being overlooked Polynucleosis is always transient and initial and never develops during the course of the attack The rise of the mononuclear reaction may overlap the fall of the polynuclear cells but in the more severe forms this reaction is delayed and the blood count becomes within normal limits and may remain so for two or three weeks or more or may fall further to a leukopenia before the mononucleosis appears Leukopenia is fairly common at the onset or during the course of severe cases before the mononucleosis develops It is mainly due to granulopenia but even the lymphocytes may fall In milder clinical types a mononuclear reaction may be present at the first examination or within a few days of onset

The monocytic and lymphocytic reactions overlap but the monocytic system subsides first and a pure lymphocytosis is finally left So rapidly may alterations take place in the types of white cells and their number and so great are the differences in different cases that no single blood picture is exclusively typical of the disease But most characteristic during the active stages is the presence simultaneously of various types of mononuclear cells particularly with a high incidence of monocytes an appearance rarely seen in any other disorder of the blood

Heterophil Antibodies

Paul and Bunnell (1932) made the curious discovery that heterophil agglutinins develop in high titer in human serum in infectious mononucleosis and in no other disease with some unimportant exceptions The development of heterophil antibodies and the technic of estimation will not be discussed

Important questions which arise are at what stage does the reaction become positive and in what proportion of cases is it positive and does a negative reaction exclude infectious mononucleosis

In the common mild types the reaction is frequently positive at the first examination which is usually four or five days after the onset If the examination is earlier the test may be negative or indefinite the titer rising in the next few days Owing to the certainty with which the diagnosis can be made on clinical and hematologic grounds the test is not always repeated Nevertheless in these circumstances the reaction is positive in nearly 90 per cent

But in the severer febrile forms the reaction may remain negative during several weeks of pyrexia and constitutional disturbances and become positive about the same time as the mononucleosis and glandular swelling develop It is striking how often the constitutional symptoms rapidly ameliorate within a few days of the rise in titer Thus the development of a positive reaction is related to the end of an attack rather than to the onset and it may well be connected with the development of immunity as Himsworth (1940) suggested On more than one occasion I have known the reaction to become positive for the first time during a relapse

The titer has no constant relationship to the severity of the disease the extent of the glandular swelling or to the degree of lymphocytosis The time during which a reaction remains positive is very variable but little more is known The titer may fall from a very high dilution to negative in the course of a few days In ordinary mild cases it may become negative within two weeks of recognition but it often persists for several weeks and has been found still positive after several months

The significance of a negative test especially arises in epidemics in which all results are reported as negative and in individual cases in which the test is repeatedly negative The question arises whether or not there are two types of infectious mononucleosis giving respectively positive and negative reactions There is nothing inherently improbable in the existence of two viruses but until we know more about heterophil agglutination the evidence must be regarded as inconclusive

I agree with the opinion of Paul and others that a positive reaction is proof of infectious mononucleosis and a negative reaction does not exclude it

Neurologic Manifestations

The neurologic manifestations of infectious mononucleosis have attracted attention recently and quite a number of cases have been recorded in the last few years It is obvious that the presence of infectious mononucleosis in similar cases must previously have been overlooked The existence of encephalitis was suspected by Longcope (1922) and by Glanzmann (1930) but the first clear descriptions of neurologic features were given by Epstein and Dameshek (1931) and by Johannsen (1931) The clinical pictures are extraordinarily varied and bizarre and no two cases appear to be quite similar The brain (encephalitis) meninges cord cranial nerves and peripheral nerves may be affected either separately or in combinations or sequences There is no constant order in which the ordinary manifestations of infectious mononucleosis and the neurologic symptoms respectively develop or in their comparative severity The glandular enlargement lymphocytosis in the blood and in the cerebrospinal fluid and meningeal or other neurologic symptoms may develop and subside simultaneously as in Epstein and Dameshek s case In other cases the infectious mononucleosis may run its course and subside to be followed by nervous symptoms lymphocytosis in the cerebrospinal fluid and a normal blood picture Or again the symptoms of a benign lymphocytic meningitis may be subsiding before the features of infectious mononucleosis appear In this last group the blood count may show an initial polynucleosis even with a high mononucleosis in the cerebrospinal fluid

The symptoms of benign lymphocytic meningitis are exactly reproduced in certain of the cases It is also noteworthy that in the more severe neurologic forms the blood changes tend to be late and the glandular swelling slight as with other severe types of infectious mononucleosis

The heterophil agglutinins have been estimated in all recorded cases since 1938 and the test always has been positive in the blood at some stage with the exception of two cases in sisters (Thelander and Shaw 1941) but it has never been positive in the cerebrospinal fluid Observers might naturally hesitate to attribute to infectious mononucleosis neurologic symptoms with negative agglutination

Recovery from neurologic manifestations takes place with extraordinary rapidity A comatose and paralyzed patient with an extensor plantar response may be apparently normal mentally and physically in three days The question of encephalitis requires further observation Severe headache is the commonest symptom in neurologic cases and is also an occasional complaint in ordinary types Children and adolescents may take a surprisingly long time six to twelve months to recover their usual powers of concentration and application after a simple attack although apparently physically normal It is possible that this is a sequel of encephalitis

Association with Jaundice

Jaundice is now not uncommon at the onset or during the course of the severer forms but the association has become frequent only in the last ten or twelve years It was not recorded in the epidemic in England in 1930 but in 1935 and 1936 many cases were observed in St Thomas s Hospital (Tidy 1937) and it is now a recognized complication When occurring at the onset the jaundice is often of considerable severity and there is nothing to distinguish the clinical condition from an ordinary infective hepatitis As the jaundice subsides the pyrexia persists and the diagnosis of infectious mononucleosis often follows the discovery of lymphocytosis in a routine blood count or the observation of some glandular swelling which occasionally is present at the onset

Jaundice adds the symptoms of infective hepatitis to the symptoms of infectious mononculeosis but does not appear otherwise to affect the course Jaundice is rarely severe when developing later in the illness Whether the jaundice is due to a separate virus cannot at present be determined

Diagnosis

Possible errors in diagnosis are numerous and mistakes in practice are not uncommon but with the transitory nature of ordinary attacks they usually settle themselves without important consequences They will not be considered seriatim

In the severer febrile forms there may be no means of establishing the diagnosis for several weeks This may also apply to onset with jaundice or with neurologic symptoms The possibility of infectious mononucleosis as the essential factor in some cases of benign lymphocytic chorio meningitis should be borne in mind The blood changes should not cause difficulty in differentiation from leukemia when the patient is seen in the acute stage In acute leukemia the toxic symptoms are always severe

An occasional but extremely difficult diagnosis may be caused by the rare slowly progressive chronic lymphoid leukemia In the earlier stages, there are periods of exacerbation with pyrexia and moderate glandular swelling The lymphocytes for several years may be only at the upper limits of normal They gradually creep up and the diagnosis long suspected slowly becomes confirmed Diagnosis is especially difficult when it is asked for on a patient some months after a pyrexial attack with lymphocytosis considered to be infectious mononucleosis Either lymphocytosis or glandular swelling may persist for several months after infectious mononucleosis but if both features are present for six months the diagnosis must be considered to be in doubt unless it has been fully established I have watched three such cases originally diagnosed doubtfully as infectious mononucleosis gradually develop into fatal lymphoid leukemia or lymphosarcoma over periods of three to ten years

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THE PATHOLOGY OF INFECTIOUS MONONUCLEOSIS

By R Philip Custer M D and Edward B Smith M D

INFECTIOUS mononucleosis is not so uniformly a benign self limited disease as is generally regarded Thomsen and Vimtrup[33] (1939) reported 6 fatalities in a series of 500 cases treated in the Blegdam Hospital in Copenhagen Two of these were complicated by some other infection but in 4 cases death occurred in uncomplicated infectious mononucleosis and was due in each instance to central respiratory paralysis The 2 *autopsies* in this group were the first we found described in the literature Jersild[14] performed an autopsy on a patient who had had angina with sepsis but who died of myocarditis attributed to infectious mononucleosis Rupture of the spleen proved fatal in the patient examined by Ziegler[41] and fatal in 4 of the 7 cases of ruptured spleens in infectious mononucleosis which we reported [9] The autopsy findings in 2 cases of Guillain Barre syndrome associated with infectious mononucleosis were described by Ricker et al [27] in 1947 *

Pathologic changes in the *spleen* following surgical removal for rupture have been reported by Atlee [2] King [16] Darley et al [7] Davis et al [8] Milne [21] Vaughan et al [38] and Smith and Custer [9] *Lymph nodes* removed during the active stages of the disease were described by Gall and Stout[1] who included a review of the literature up to 1940 Moeschlin [4] presented his findings on aspiration biopsies performed in 3 cases (puncture of lymph nodes in all 3 of sternum in 2 and of spleen in 1) The *bone marrow* was erroneously described by Freeman [11] but Nordenson [5] and Limarzi Paul and Poncher[17] have given accurate accounts of the changes in this tissue A biopsy of the *liver* performed at the time of splenectomy was illustrated by Davis et al [8] and aspiration biopsies have been discussed by Kilham and Steigman [15] van Beek and Haex [37] and Bang and Wanscher [3]

The aggregate of the findings in this group of scattered case reports is in essential agreement with our observations in the following series although with few exceptions the descriptions have been incomplete and the tissue changes inadequately illustrated Moreover the diagnostic value of histologic appearances in the lymphatic organs has usually been underestimated The purpose of this paper is to present as nearly a complete pathologic picture of infectious mononucleosis as possible

Material

This study was based on the following material most of which was observed while the authors were on duty at the Army Institute of Pathology during the recent war

Autopsies	9
Lymph node biopsies	100 plus

From the Laboratories of the Presbyterian Hospital in Philadelphia and the Army Institute of Pathology Washington D C

* These are our Cases 1 and 2 which we retained in our series because Ricker et al gave attention more particularly to the neurologic feature

Bone marrow a pirates	25 plus
Bone marrow biop ies	2
Extirpated ruptured spleens	3
Tonsillar tumor	1
Liver biopsy	1
Skin biopsies	2
Causes of death in the fatal ca es w re as follows	
Spontaneous rupture of spleen	4 ( )
Guillain Barré syndrome	2
Nasopharyngeal hemorrhage	1
Laryngeal edema	1
Airplane accident (convalescent ca e)	1

Pathologic Findings

Repeated reference will be made to the atypical or abnormal lymphocyte the so called infectious mononucleosis cell We described this cell in a previous paper[29] as follows When stained lightly with hematoxylin and eosin in thin sections it varies from 12 to 15 microns in diameter occasionally larger and is round except when distorted by crowding The cytoplasm is homogeneous and faintly acidoph ilic The centrally or eccentrically placed nucleus is sharply delineated by a thin membrane which blends with the marginal chromatin particles chromatin is irregularly distributed to lend a mottled appearance and it occasionally forms an gulated bars Indentation and folding of the nuclei can be demonstrated in rela tively few cells It is virtually impossible to determine in the sectioned material whether a true nucleolus is present or not nor could fenestration be evaluated We regard these cells as atypical or abnormal lymphocytes closely related to if not identical with those found in the peripheral blood

Following common usage we have employed the terms infiltration and in filtrate with reference to the presence of normal and atypical lymphocytes in con nective tissues and as perivascular collars i e situations where they are not nor mally found We regard the majority of these cells however as of local origin and probably derived from pre-existing cells of the reticulo-endothelial system

Hematopoietic System Lymph nodes were usually but not invariably enlarged and displayed a variety of histologic appearances In some instances follicles were well preserved (fig 1) and frequently assumed rather striking proportions (this is prob ably a transient phase) The structure of the follicular centers was not significantly altered except for a scattering of abnormal lymphocytes histiocytes often contained cellular debris and mitotic figures were common findings In nodes such as these the sinus structures were well preserved and contained varying numbers of normal and abnormal lymphocytes (fig 2) The medullary cords were richly cellular and the same lymphocyte admixture was noted here In a lesser number of cases the architectural pattern of the node was blurred follicles being inconspicuous or even absent and the sinus tracery was obscured as result of lymphocytic and reticulo endothelial hyperplasia (fig 3) This change was occasionally so striking as to

The immediate cau es of death in these 4 cases vere hemorrh ge (2) postoperative pulmonary embolism (1) blood transfusion react on (1) In 3 other cases the r ptured spleen was removed and the patients recovered

Table 1 —*Distribution of Lesions in Nine Fatal Cases of Infectious Mononucleosis*
(+ distinct lesion ± minimal lesion — no lesion blank no tissue)

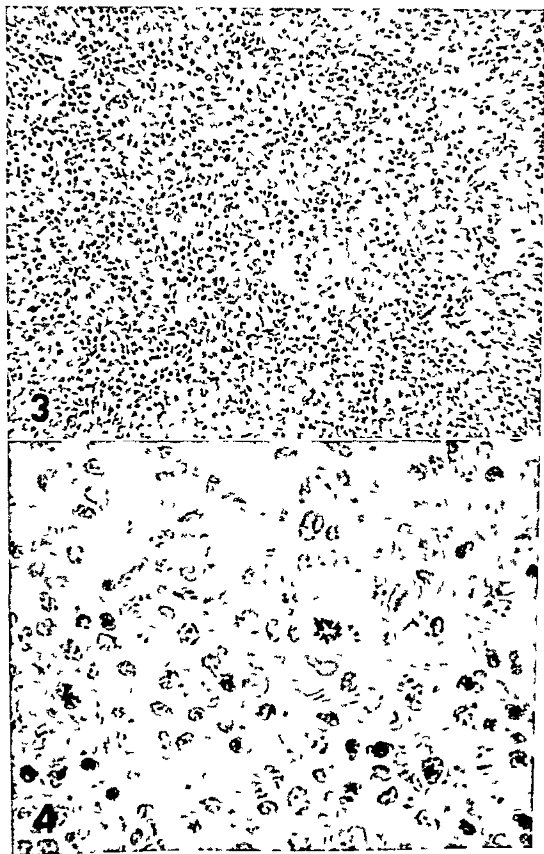
	Case No								
	1	2	3	4	5	6	7	8	9
	AIP N								
	163849	151166	146828	67260	149279	151263	103388	944 5	131476
Age	21	22	23	21	24	22	21	26	20
Sex	M	M	M	M	M	M	M	M	M
Color	W	W	W	W	W	W	W	W	W
Day of Disease	22+	17	30	14	?	13	33	35?	17
Hematopoietic System									
Lymph Nodes	+	+	+	+	+	+			+
Spleen ( )	+	+	+	+	+	+	+	+	+
Bone Marrow	—	—	—	—	—	—		—	
Respiratory System									
Paranasal Sinuses				±					
Pharynx (incl tonsils)	+					+			
Tracheo bronchial Tree	+								
Lungs	+	+	±	±	—	±	±	±	+
Cardiovascular System									
Heart	—	+	+	+	+	+	±	+	
Aorta		+	—			—			
Peripheral Vessels	+	+	+	+	+	+	+	+	+
Digestive System									
G I Tract									
Stomach	+		±	+	+				
Intestine	±	±	+		±				
Liver		+		+	+	+	+	±	+
Pancreas	—	—		—		+			
Genitourinary System									
Kidneys	+	+	+	+	—	+	+	—	
Lower Urinary Tract			+	+					
Prostate	+		+	+					
Testes	+		+						
Endocrine System									
Adrenal	+	+	±	—	±	+	+	+	
Thymus	±		±						
Pituitary		+	+	±	—				
Nervous System									
Meninges	±	+	+	±			—		
Brain	+	+	+	+	—		+		
Spinal Cord	+	±							
Peripheral Nerves	+	+							
Musculature		—	+						
Integument	(None from autopsies two biopsies positive)								
Weight of Spleen (gm)	675	60	150	355	(4x)	(3x)	4 0	(4x)	†
Weight of Liver (gm)	2100	2600	1650	†	†	†	†	2738	†

Cause of Death *Case 1* Respiratory paralysis (Guillain Barre syndrome) *Case 2* Respiratory paralysis (*Guillain Barré* syndrome) *Case 3* Airplane crash *Case 4* Hemorrhage from ruptured spleen *Case 5* Hemorrhage from ruptured spleen *Case 6* Edema of glottis *Case 7* Lower nephron syndrome (transfusion post splenectomy) *Case 8* Pulmonary embolism (post splenectomy) *Case 9* Hemorrhage from nasopharynx

† No weight given



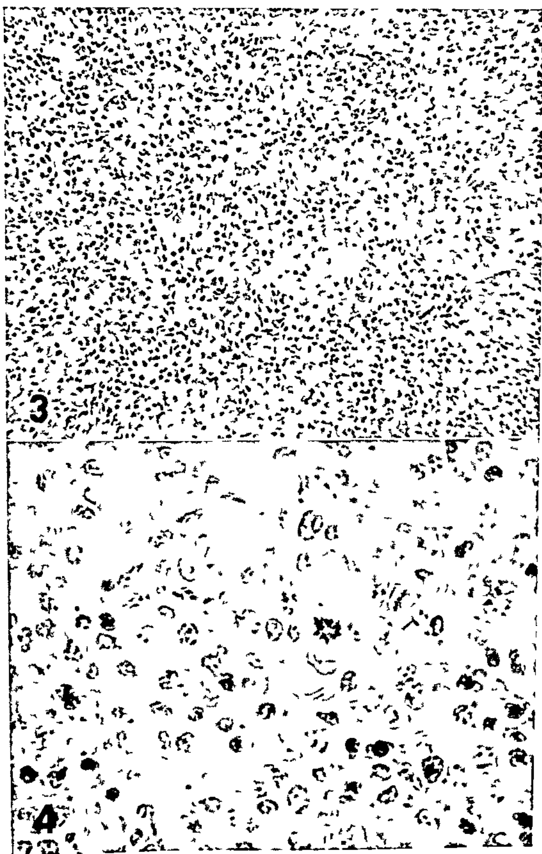
Figs. 1-2 (S[illegible]



Figs 3 4 (See p 20)



Figs 5 6 (See p 720)



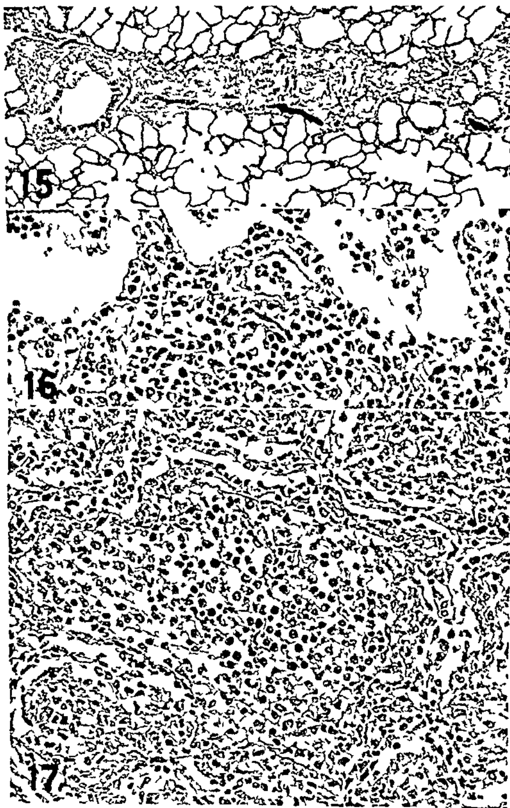
Figs 3 4 (See p 720)



Figs 9–11 (See p 720)



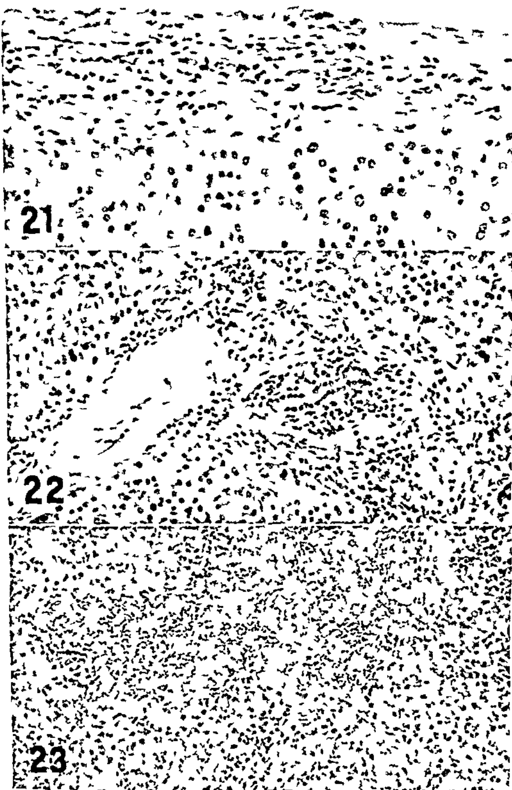
FIGS 7 8 (See p 720)



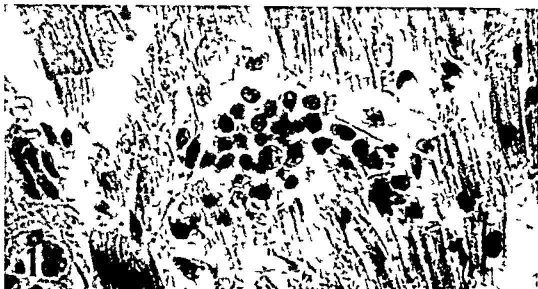
Figs 15 (See p 720)



Figs 12 14 (See p 20)



FIGS 21–23 (See p. 721)







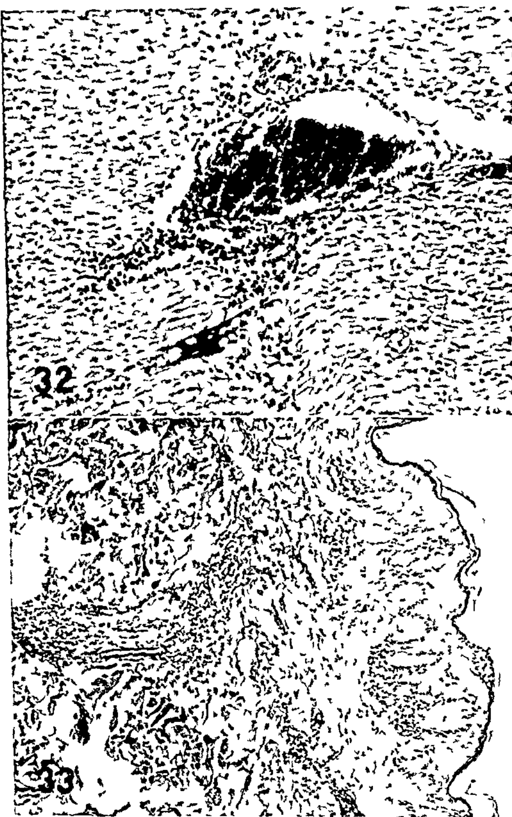
FIGS 8 2 (See p -2)



F GS 27 29 (See p 721)



Fi 4 6 Nc [21



Figs. 32, 33 (See p. 721)

30

31

Figs 30–31 (See p 721)

FIG 18 HEART Focus of normal and atypical lymphocytes in the interstices of the heart in most instances such collections are perivascular in position Slight edema is occasionally noted but muscle fibers show no structural changes (× 800)

FIG 19 STOMACH An increased number of lymphocytes in the tunica propria There is also a conspicuous zone of round cells on the deep margin of the muscularis mucosa (× 100)

FIG 20 ILEUM Peyer s patches are moderately hyperplastic (one case) (× 30)

FIG 21 LIVER Capsule displays a lymphoid reaction similar to that observed in the spleen The connective tissue appears to be losing substance in proportion to the increase in lymphocytes (× 350)

FIG 22 LIVER Minimal lymphocytic infiltration of the periportal connective tissue (× 200)

FIG 23 LIVER Marked periportal lymphocytosis maximal in our series simulating lymphoid leukemia Lymphocytes are also conspicuous in surrounding sinusoids (× 225)

FIG 24 KIDNEY Lymphocyte accumulations in the interstitium of the cortex (× 350)

FIG 25 KIDNEY Perivenous aggregates of lymphoid cells in the boundary zone (× 200)

FIG 26 ADRENAL Perivenous collections of lymphocytes indistinguishable from those frequently encountered in any routine autopsy series (× 350)

FIG 27 THYMUS Adventitial reaction around a small artery cut tangentially predominantly atypical lymphocytes (× 50)

FIG 28 PITUITARY Rather extensive interstitial collections of lymphocytes (× 350)

FIG 29 MENINGES Pia arachnoid showing patchy round cell aggregates chiefly in the neighborhood of blood vessels (× 1000)

FIG 30 BRAIN Lymphocytic infiltration in the periphery of a moderate sized blood vessel There is edema of the adjacent tissue (× 200

FIG 31 BRAIN Perivascular cuffing and edema resembling that seen in various forms of virus encephalitides (× 450)

FIG 32 ANTERIOR SPINAL NERVE ROOT Marked perivascular lymphocytic reaction in a case of infectious mononucleosis with Guillain Barré syndrome Appropriate stain demonstrated degeneration of myelin and distortion of axis cylinders of the nerve fibers (× 200)

FIG 33 SKIN There is hyperemia and edema of the corium associated with round cell reaction about vessels and appendages (× 100)

simulate a malignant lymphoma (fig 4) Mitotic figures were abundant but no atypical mitoses were observed Connective tissue of the lymph node capsule trabeculae and hilum were more or less infiltered with round cells comprising for the most part both normal and atypical lymphocytes The lymph sinuses in the hilum and perinodal tissue were usually crowded with similar cells (figs 5 and 6)

The *spleen* was invariably enlarged during the height of the disease as shown by the weights in our cases (table 1) the one normal weight of 150 Gm in our series was in a convalescent patient who was accidentally killed Milne [1] mentioned a 970 gram spleen which ruptured and was successfully removed The histopathology of the spleen has been recorded in detail in a previous article [9] To summarize briefly the capsule showed varying degrees of infiltration with round cells consisting largely of both normal and abnormal lymphocytes The trabeculae displayed dilution of the fibromuscular structure by a similar infiltrate (fig 9) not infrequently to the point at which complete dissolution occurred the site of the trabeculae in such instances was marked only by the trabecular vessels Follicles were usually widely spaced in some instances being well preserved and hyperplastic in others existing merely as ill-defined aggregates of small lymphocytes (fig 8 Occasionally the eccentric arteriole was the only landmark The blood

FIG 1 LYMPH NODE A representative field from a node in which the general pattern was preserved The follicle illustrated is rather poorly defined but shows active proliferation in the follicular center and lymphocytic hyperplasia in the periphery The sinusoid in the upper left is choked with normal and atypical lymphocytes Reticulum cell outcropping is noted in the lower right and the trabecula to the right displays a lymphocyte reaction (× 200)

FIG 2 LYMPH NODE A medullary cord at higher magnification presents hyperplasia of lymphocytes reticulum cells and lining endothelium of a sinus The sinus contains normal and atypical lymphocytes one of the latter lying outside of the sinus in the upper center (× 1000)

FIG 3 LYMPH NODE An extraordinary hyperplasia observed in a node removed during the peak of the *illness There is a striking hyperplasia of reticulum cells and abnormal lymphocytes to a degree that the* entire nodal pattern is virtually obliterated to give the impression of a malignant lymphoma The sinus *tracery is preserved however marked by compact circumscribed aggregates of small lymphocytes* (× 200)

FIG 4 LYMPH NODE Higher magnification of the node shown in Fig 3 emphasizing the resemblance to a malignant lymphoma of the reticulum cell type True nucleoli are small and not so conspicuous as irregular chromatin clumps and angulated bars Mitoses are plentiful but never atypical (× 1000)

FIG 5 LYMPH NODE Section through periphery showing an afferent lymph vessel on the left the lumen containing large numbers of normal and abnormal lymphocytes The peripheral sinus of the node proper (right center) is also distended with round cells and the capsule has virtually lost its identity by virtue of the lymphocyte reaction in the connective tissue this is somewhat more marked than is usual (× 200)

FIG 6 LYMPH NODE Section through hilum disclosing increased cellularity of the interstices and *packing of efferent lymph channels with lymphoid elements even more marked than one finds in the* capsule (× 200)

FIG 7 BONE MARROW There is a moderate granulocytic hyperplasia and a relative immaturity of the cells of this series The small round cells shown in the picture are erythrocyte progenitors Megakaryocytes are increased in number in proportion to the granulocytic hyperplasia No lymphoid aggregates are present (× 1000)

FIG 8 SPLEEN View at low magnification demonstrating a blurred pattern except for prominent subcapsular blood sinuses the sinuses usually contain clumps of normal and atypical lymphocytes The capsule and trabeculae show dilution and partial dissolution by reason of lymphocytic infiltration *In the lower right is a small poorly defined Malpighian follicle in other cases the follicles are large with* prominent germinal centers (× 30)

FIG 9 SPLEEN A stage in the dilution and dissolution of trabeculae as result of lymphocytic infiltration (presumably metaplasia of the trabecular connective tissue) (× 200)

FIG 10 SPLEEN Subendothelial lymphocytic zone in trabecular veins (× 200)

FIG 11 SPLEEN Adventitial lymphocytic reaction around intratrabecular artery (× 200)

FIG 12 TONSIL Extensive necrosis with small island of residual lymphoid tissue Necrosis of this degree is infrequent and follows ulceration and secondary infection Lack of neutrophil response is common but inconstant (× 200)

FIG 13 TONSIL Section through tonsillar bed discloses a marked lymphocytic reaction in the capsul (right) and in the interstices of an adjacent mucous gland (left) (× 200)

FIG 14 TONSIL Section through a rapidly developing unilateral tonsillar tumor occurring during the acute phase of infectious mononucleosis As in the lymph node illustrated in Figs 3 and 4 the histologic appearances simulate those of a malignant lymphoma (heterophil titre 1 1260 complete recovery) (× 1000)

FIG 15 LUNG Peribronchial connective tissue and interlobular septum heavily infiltrated with lymphoid cells *the process involves interalveolar septal walls rendering them thicker and more prominent* (× 30)

FIG 16 LUNG Extensive interstitial pneumonia with virtually no intra-alveolar exudate the cellular reaction is entirely lymphocytic (× 450)

FIG 17 LUNG Lobular pneumonia showing a rich fibrin network supporting a cellular exudate which is almost entirely of lymphoid type (In another area neutrophils predominated) (× 450)

serosal reaction respectively except in case 3 where the cellular infiltrate was rather prominent in some areas No real pericarditis was encountered The aorta was examined in 3 cases It was quite normal in 2 patients who died respectively on the thirteenth and thirtieth day of the disease but in the third, who lived seventeen days perivascular cuffing was seen about the vasa vasorum in the adventitia the cells being the usual normal and atypical lymphocyte varieties

As regards the *peripheral vessels* one may generalize that apart from the lymphoid tissues proper the lymphocytic proliferation was either adventitial or subintimal in location irrespective of the organ or tissue examined The arterial involvement tended to be adventitial whereas the venous was subintimal

Digestive System The tunica propria of the *stomach* contained collections of lymphoid cells considerably in excess of normal (fig 19) in 3 of the 4 cases examined In the fourth case a convalescent patient who had been ill 30 days the reaction was equivocal Sections of *ileum* from 4 cases proved difficult to evaluate because of the variable prominence of lymphoid tissue under normal circumstances in this age group The lymphoid proliferation was distinctly abnormal in only 1 of these cases (fig 20) and the cellular reaction was that of the lymphoid tissues elsewhere

Liver changes were uniform qualitatively in the specimens examined from 7 autopsies and 1 biopsy The capsule in most instances showed varying degrees of involvement similar to that seen in the spleen (fig 21) The lymphocytic infiltration was most pronounced in the periportal connective tissue Quantitatively this reaction varied from hardly more than the usual lymphocyte collar (fig 22) to a degree approaching that of lymphatic leukemia (fig 23) with lymphoid cell aggregates extending into the adjacent lobular parenchyma In these latter instances there was an apparent loss of some of the peripherally placed liver cells associated with minor bile duct proliferation Necrosis of the liver parenchyma was not observed in our series except in 1 case where portal vein thrombosis followed splenectomy There was no evidence of biliary obstruction Those cases showing a lymphocytic leukocytosis also displayed considerable numbers of round cells throughout the lobule but within blood sinusoids This more diffuse intralobular cellularity was augmented by Kupffer cell hyperplasia

Perivascular lymphocytic collars were noted in only 1 of the 4 cases in which sections of the *pancreas* were available for study The parenchyma of the organ was apparently normal

Genito-Urinary System The *kidneys* were involved in 6 of 8 cases In common with the other tissues the lesion was confined largely to the periphery of blood vessels notably the subintimal zone of the larger veins in the columns of Bertini (fig 25) Aggregates of lymphocytes were also found in the interstices of the cortex (fig 24) There were no alterations in the nephrons except in the case of a transfusion reaction where a hemoglobinuric nephrosis (lower nephron nephrosis) was present

The 2 sections of *urinary bladder* 3 of *prostate* and 2 of *testis* all presented interstitial foci of lymphocytes in the neighborhood of small blood vessels

Endocrine System The *adrenal* was examined in 7 cases 5 of which displayed clusters of normal and abnormal lymphocytes beneath the intima and in the periphery of the central vein (fig 26) as well as in the capsule The occurrence of lymphocytes

sinuses of the spleen contained more nucleated cells than erythrocytes except when there was marked congestion of the organ The nucleated forms again were mostly normal and atypical lymphocytes The red pulp likewise contained a predominance of these cell types The changes in the blood vessels were twofold first an adventitial infiltration of lymphoid elements around intratrabecular arteries (fig 11) and second a similar subendothelial infiltration of the veins (fig 10) These vascular findings have been noted also in leukemias and scarlet fever occasionally in chronic malaria and acute fulminating infections The subintimal infiltrate in veins has also been noted in allergic states, especially drug sensitivity in which the patient survived more than twenty four hours

The *bone marrow* contained no abnormal cells apart from those in the circulating blood (fig 7) The marrows were either quite normal or in some instances moderately hyperplastic Hyperplasia was usually limited to the granulocyte series although megakaryocytes occasionally seemed more numerous than usual Unfortunately in our Case 4 characterized by purpura and thrombocytopenia no bone marrow sections were obtained

Respiratory System Mucous membranes of the sphenoid and ethmoid sinuses were available for study in 1 case These appeared normal apart from a minor submucosal round cell infiltration which was nonspecific in character

The *tonsils* were examined in 2 of the autopsied cases Both showed areas of necrosis and a reaction of normal and abnormal lymphocytes with a variable admixture of plasmocytes and neutrophils (fig 12) The round cell infiltration was also noted in the peritonsillar tissue (fig 13) In 1 case the lingual tonsils and posterior pharyngeal wall were sectioned and displayed a similar cellular reaction deep in the striated muscle and around mucous glands of the tongue and pharyngeal wall The *larynx* and *trachea* in another showed a like mononuclear cell infiltrate In a patient who recovered a rapidly growing tonsillar mass was regarded as a tumor and removed surgically The tissue presented an amazing proliferation of lymphoid and reticulo endothelial elements to a degree mimicking reticulum cell sarcoma (fig 14) the surface was ulcerated and the obvious inflammatory reaction was limited to the margin of the ulcer

The *lungs* displayed a variety of appearances In 2 instances there was a frank pneumonic consolidation the cellular exudate in 1 was of the classic polymorphonuclear neutrophil type while in the other it was made up almost exclusively of large lymphoid cells (fig 17) Most cases showed a more or less marked exaggeration of the peribronchial and bronchiolar collar and in some the round cell reaction extended along the intra alveolar septa to simulate interstitial pneumonitis (figs 15 and 16) Cluster of lymphoid cells were also noted in the subpleural connective tissue

Cardiovascular System In 6 of the 8 cases in which the *heart* was examined histologically aggregates of lymphocytes were sparsely distributed within the myocardium in the periphery of small blood vessels (fig 18) They were also present in small numbers beneath the endocardium In 1 case the reaction was virtually negligible and in another completely absent in the tissue studied The myocarditis and pericarditis were minimal and unassociated with any necrosis of muscle or

Table 2.—[illegible] Diagnoses

Hematopoietic System	
Infectious mononucleosis	37
Lymphadenitis	10
Respiratory System	
Epistaxis	4
Acute sinusitis	1
Nasopharyngitis	45
Acute pharyngitis	64
Acute tonsillitis	36
Bronchitis	10
Atypical pneumonia	19
Influenza	4
Digestive System	
Vincent's angina	5
Gastro-enteritis	3
Jaundice	6
Nervous System	
Psychoneurosis	1
Suspected meningitis	3
Heat exhaustion	2
Miscellaneous	
Malaria	[illegible]
Reaction to typhoid vaccine	1
Cervical mass	[illegible]
Cervical myositis	5
Diagnosis uncertain	12

Table 3.—Admission Complaints

Hematopoietic System	
Lymphadenopathy	81
Respiratory System	
Epistaxis	6
Sore throat	146
Cough	39
Digestive System	
Anorexia	58
Nausea	9
Vomiting	[illegible]
Abdominal pain	14
Jaundice	6
Nervous System	
Headache	[illegible]
Vertigo	9
Miscellaneous	
Malaise	[illegible]
Fever	6
Arthralgia	[illegible]
Myalgia	5
Cutaneous eruption	11

in the adrenal is so common in the average series of autopsies that it is difficult to evaluate this finding as related to infectious mononucleosis

The lymphoid tissue of the *thymus* was not hyperplastic in either of the 2 cases examined and the organs were not enlarged grossly In one of the glands there was a perivascular reaction in which abnormal lymphocytes were present (fig 27) the findings in the other being equivocal

Two of the 4 *pituitary glands* displayed prominent aggregates of lymphocytes in the interstices (fig 28) a third showing a few such cells and the fourth being essentially normal

Nervous System The central nervous tissues and peripheral nerves in our Cases 1 and 2 have been described in detail by Ricker et al [7] and adequately illustrated In summary the meninges were congested and edematous and contained moderate numbers of mononuclear cells (fig 29) Occasional small perivascular hemorrhages were noted within the brain substance as well as mild ganglion cell degeneration occasionally with satellitosis Changes in the spinal cord were minor but cellular infiltration of anterior nerve roots was noted at all levels particularly in the periphery of blood vessels (fig 32) The myelin sheaths were swollen and disrupted In 3 other cases distinct perivascular cuffing with round cells had occurred in the brain (figs 30 and 31) and in 2 of these the meninges were similarly affected An additional case displayed no demonstrable involvement Thus in 5 of 6 cases there was evidence of a mild to moderate meningo encephalitis and in 2 a distinct peripheral neuritis

Musculature Sections of voluntary muscle were examined in 2 cases one appearing normal the other showing lymphocytic collars in the periphery of small blood vessels

Integument Two biopsies of skin revealed a relatively normal epidermis save for a lymphocytic infiltration of some of the rete pegs (fig 33) The corium was rather edematous and hyperemic and an infiltration of small and large lymphocytes was noticeable in the vascular peripheries

Clinico Pathologic Correlations

We have shown that there are as many lesions of infectious mononucleosis as there are organs and tissues of the body although the degree of involvement of each varies markedly from case to case This is manifest clinically by the wide range of signs and symptoms listed in the many series of cases reported in the literature We have selected Read and Helwig s [6] analysis of 300 Army cases to illustrate this and to show that virtually all of the clinical features may be explained on the basis of demonstrable pathologic changes Tables 2 3 and 4 are taken directly from their article although the items have been rearranged by systems to conform with the scheme used for our pathologic descriptions In the following paragraphs we will also add certain features of the disease not mentioned in Read and Helwig s series It is interesting to note that most of the clinical manifestations of the disease serve to confuse rather than to clarify the diagnosis

Hematopoietic System In view of the fact that hyperplasia of lymphoid tissue is the major and most consistent pathologic change in infectious mononucleosis it is

lowing rupture of the spleen or with associated pyogenic infection may occur very rapidly and demonstrates the responsiveness of the marrow sometimes masking the original lymphocytosis

Minot [3] was the first to describe the striking thrombocytopenia with hemorrhagic purpura which occasionally complicates infectious mononucleosis and then regresses during convalescence As Minot s[2] case was observed before the heterophil antibody reaction had been described he hesitated to make a precise diagnosis but in view of recent reports[15, 20, 30] there can be no doubt that this was infectious mononucleosis One of our patients (Case 4) who died following rupture of the spleen had a condition of this type but unfortunately bore marrow was not removed at autopsy The only marrow study of the thrombocytopenic variant which we have found was reported by Dameshek and Grassi[6] whose patient recovered after splenectomy they described an increase in megakaryocytes which showed greatly diminished platelet production

Respiratory System The essential lesion in the nose and throat is lymphoid hyperplasia which may subside uncomplicated by secondary infection or necrosis and without undue discomfort In other instances however, ulceration supervenes with extension of secondary invaders to produce membranous pharyngitis peritonsillar abscesses and even Ludwig s angina Epistaxis probably results from ulceration and necrosis of hyperplastic lymphoid tissue in the nose tissue was not available for study in our Case 9 in which death was due to nasopharyngeal hemorrhage so that we were unable to demonstrate this However in Case 6 there was actual gangrene of the tonsil and posterior pharyngeal wall and in Case 1 necrosis of tonsils to a lesser degree Occasionally the lymphoid overgrowth may be so exuberant as to simulate a malignant tumor as in one of our patients who made an uneventful recovery

Tidy[36] and Wintrobe[40] state that laryngeal obstruction is unknown yet the cause of death in our Case 6 was edema of the glottis Cough is probably due most commonly to tracheobronchitis observed in the single specimen available to us and to actual involvement of lung tissue which was slight in 5 of our 9 autopsies prominent in 3 and absent in 1 It is interesting that 19 of Read and Helwig s [6] cases were diagnosed atypical pneumonia on admission figs 15 and 16 make clear that interstitial pneumonitis actually can occur as a feature of infectious mononucleosis and would be indistinguishable clinically and by x ray examination from the interstitial pneumonitis of atypical pneumonia influenza or even rheumatic pneumonitis Similarly the lobular pneumonia found in Case 9 can be linked to infectious mononucleosis only by the mononuclear exudate in the alveoli The sometimes marked acceleration in respiratory rate is probably due to pneumonitis or pneumonia usually not recognized as part of infectious mononucleosis per se

Cardiovascular System No symptoms referable to the heart are listed in Read and Helwig s[26] article However arrhythmias and tachycardia are known to occur Wintrobe[40] mentions one patient in whom tachycardia and cyanosis became so pronounced that acute cardiac dilatation was suspected Myocarditis was the stated cause of death in Jersild s[14] case

Electrocardiographic evidence of heart lesions has been reported In Evans and

readily understood why enlargement of the *lymph nodes* and *spleen* are the most commonly observed physical findings The rapidity with which the enlargement develops accounts for the tenderness of these organs We have shown in a previous article [9] that the tense swollen spleen of infectious mononucleosis is peculiarly liable to rupture because the capsule and trabecular are more or less diluted and sometimes dissolved by lymphocytic infiltration

TABLE 4 —*Positive Signs*

Hematopoietic System	
Generalized adenopathy	172
Cervical adenopathy	123
Tender adenopathy	67
Palpable spleen	104
Tender spleen	39
Petechiae	9
Oral cavity	4
Generalized	9
Respiratory System	
Epistaxis	6
Follicular pharyngitis	112
Membranous pharyngitis	34
Acute tonsillitis	29
Peritonsillar abscess	7
Hemoptysis	3
Digestive System	
Gingivitis	37
Vomiting	7
Diarrhea	4
Palpable liver	47
Jaundice	11
Abdominal tenderness	3
Nervous System	
Stiff neck	2
Mild stupor	3
Delirium	1
Miscellaneous	
Myositis	5
Dermatitis	16

Erythropoiesis in the *bone marrow* in nearly all cases appears to be essentially normal thus explaining the rarity of anemia in infectious mononucleosis The cause of anemia in the few cases reported was not apparent in other instances the anemia was regarded as coincidental Granulocyte components are either normal or increased in number and may show some degree of immaturity and toxic granulation (Limarzi et al) Study of cases with actual neutropenia has been inadequate to demonstrate any significant change in the marrow Neutrophilic leukocytosis fol

cases apparently occurring as an isolated phenomenon it may also appear as part of the general hemorrhagic manifestations in the thrombocytopenic variant The renal lesion of infectious mononucleosis is essentially an interstitial nephritis As for the genitalia uterine bleeding in the hemorrhagic form of the disease is the only symptom of which we are aware in Dameshek and Grassi s[6] case there was also a suggestion of a pre existing hemorrhagic tendency

Nervous System Although Read and Helwig [4] do not mention headache in their series this is a very common symptom often associated with blurring of vision and vertigo and occasionally with stiff neck Stupor coma delirium and convulsions have been observed as well as paresthesias motor paralyses and depression of the respiratory center Two of our fatal cases presented the Guillain Barre syndrome A wide variety of neurologic signs has been reported [12 2 25 3] The cerebrospinal fluid findings are variable more frequently being normal the pressure is sometimes increased and a lymphocytosis of several hundred cells and increase in protein have been observed The differential diagnosis between infectious mononucleosis and lymphocytic choriomeningitis may be difficult under such circumstances Tidy[35] has attempted to relate the two diseases but Viets and Warren[39] state that in lymphocytic choriomeningitis all organs except the central nervous system are relatively normal and describe inclusion bodies in ganglion cells more extensive meningeal and perivascular lymphocytic infiltrations perivascular hemorrhage and glial nodules

We have demonstrated varying degrees of encephalitis meningitis or both in 3 of 4 cases in which no symptoms referable to the central nervous system were recognized However one of these patients lost his life in the crash of an airplane which he was piloting during the convalescent period and the disease may have been a factor leading to the accident In our 2 cases presenting the Guillain Barre syndrome there was the additional involvement of spinal nerve roots but only minor ganglion cell changes in the cord proper peripheral nerves were examined in 1 of these cases and showed lesions similar to those of the nerve roots

Musculature Myalgia is an uncommon symptom although we found myositis in 1 of 2 cases in which skeletal muscle was examined

Integument Cutaneous eruptions of various types usually morbilliform are frequently encountered in infectious mononucleosis [31] Our 2 biopsies from such cases disclosed hyperemia edema and cellular reaction in the corium

SUMMARY

This pathologic study is based on 9 autopsies and many biopsies in cases of infectious mononucleosis

The gross changes were almost exclusively confined to enlargement of lymphoid tissues especially the spleen Nasopharyngeal lymphoid hyperplasia was constant, in one instance suggesting tumor Other tissues presented no significant gross features related to the primary disease except for (1) rather consistent enlargement of the liver (2) infrequent icterus and (3) occasional cutaneous rash Histologic observations revealed more or less generalized lesions resembling those of certain known virus diseases notably perivascular aggregates of normal and abnormal lymphocytes Reaction of this type inconstantly involved all tissues studied except

Graybiel s[10] 4 cases the T waves were lowered or inverted in all leads suggesting pericardial involvement with gradual return to normal during convalescence Logue and Hansen[19] record first degree heart block with prolonged P R interval while Candel and Wheelock s[4] case had electrocardiographic tracings indicative of acute myocarditis

We were unable to demonstrate pericarditis in our 9 cases at most there was a sparse sprinkling of lymphocytes in the subepicardial connective tissue no greater than that one frequently encounters in a series of routine autopsies Lesions were found in the muscle however in 6 cases doubtful in 1 and in Case 9 no heart sec tions were available for study The foci were small in all but Case 3 where there were rather extensive residual areas of myocarditis at the time of accidental death thirty days after the onset of illness

Digestive System It is virtually impossible to demonstrate an anatomic basis for the nausea, vomiting and diarrhea occurring in a febrile illness We did find lymph ocytic infiltration of app e iable degree in the tunica p op ia of the stomach in 3 of 4 cases examined and fairly marked hyp rplasia of intestinal lymphoid tissue in 1 of 4 cases this change being equivocal in 3 It is doubtful whether these observa tions are of clinical significance

Abdominal pain and tenderness are probably due to rapid enlargement of the spleen liver and abdominal lymph nodes

Physical examination of patients with infectious mononucleosis frequently dis closes an enlarged liver In our 4 cases in which figures were recorded the organ weighed 1650 Gm 2100 Gm 2600 Gm and 2738 Gm The normal sized liver was from a convalescent case whereas the patient with the largest liver died from a pulmonary embolism five days after operation for ruptured spleen on approximately the thirty fifth day of the disease It should be mentioned that hepatomegaly is not usually a feature of the milder case

Liver fun tion tests repo ted by Cohn and Lidman[5] in a series of 15 consecutive cases of proven infe tious mononucleosis without jaundice gave evidence of liver damage they found the thymol turbidity and bromsulfalein excretion tests of most consistent value There app ared to be a rough correlation between the severity of the disease and the degree of hepatic impairment Their results were confirmed by DeMarsh and Alt[9] in 19 additional cases Jaundice is not a rare manifestat on of infectious mononucleosis and provides further evidence of liver involvement Changes in the liver in jaundiced cases as described by Bang and Wanscher[3] do not differ from those in patients who have not been jaundiced They found no reason to regard the jaundice as due to ob truction

The liver lesion is e sentially a p riportal hepatitis It is hardly distinguishable from liver change in the milder cases of epidemic hepatitis as observed at biopsy Necrosis of liver cell was observed in only 1 of our fatal cases and was apparently due to po al vein thrombo is in ident to splenectomy rather than to infectious mononu leo is p r se There may however b some slight loss of liver cells in the p riphery of heavily infiltrated portal areas

Genito Urinary System Albuminuria is common as in any febrile disease leuko cytes and red blood cells are not infrequent urinary findings but casts are not gen erally seen Gross hematuria was found in 6 p r cent of Tidy and Morley s[31] 270

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the bone marrow here lymphocytes were virtually absent in sections but were present in aspirated marrow because of dilution with peripheral blood

More specific changes were invariably noted in lymphoid tissues The abnormal lymphocyte characteristic of the disease could be identified in thin lightly stained sections Lymph node reactions varied from a predominantly follicular hyperplasia to a blurred pattern simulating a malignant lymphoma the latter was due to a lymphocytic and reticulo endothelial proliferation in the medullary cords The spleen displayed a lymphocytic infiltration in the thinned capsule and trabeculae frequently dissolving the latter and rendering the organ liable to rupture The pattern was partially effaced in most instances and the follicles widely spaced Blood sinuses contained considerable numbers of normal and abnormal lymphocytes and accumulations of these cells constantly cuffed intratrabecular arteries and lay beneath the intima of veins Tonsils displayed ulceration and necrosis in several cases and the lymphocytic proliferation closely resembled malignant tumor in a tonsil that enlarged rapidly

A pneumonic exudate in 1 case was almost exclusively of round cell type while in another the pneumonia was of the usual lobular type with a neutrophilic exudate Small myocardial infiltrates which we noted probably explain the electrocardiographic changes described in infectious mononucleosis Other findings of particular interest were the periportal lymphoid collars in the liver which sometimes attained the proportions seen in leukemia and the presence of meningo encephalitis in 4 of the 6 brains examined

We believe that the majority of cells in the lymphocytic infiltrates of connective tissues and the perivascular collars are metaplastic rather than inwandering i e that they are formed in situ and stem from cells of the reticulo endothelial system

ADDENDUM

Since this paper was prepared our Case 3 has been reported separately by Allen and Kellner [1] We have held the case in our series however as it lends strength to our tabulated findings

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were a slightly enlarged spleen about 14 to 16 centimeters occasionally palpable on deep inspiration depending on the patient s body build The spleen could be outlined by direct percussion (not by the intermediate method) by using very light tapping It could be demonstrated by x ray examination The enlarged spleen elevated the cardiac apex while the patient was lying down so that the cardiac tip was from 10 to 11 cm to the left of the midline On standing the apex lowered to about 9 cm to the left of the midline

The second feature noted in most of the patients was the comparatively low blood pressure The systolic figures were from 90 to 105 occasionally as high as 115 with diastolic pressures of 56 to 70 This was significant as most of the patients belonged to the older age group There were no definite signs of myocardial or circulatory insufficiency and there was usually no edema of the ankles

In most of the patients who made observations on their temperature there was an afternoon rise to 99 6–101 4 F Rarely was it higher than this but in some the elevation was so persistent that they had been diagnosed fever of unknown origin or were suspected of having undulant fever Hodgkin s disease or tuberculosis

Most of the patients had some lymph nodes which were palpable but never very large Enlarged posterior cervical nodes were the most common In many the nodes were well within normal limits of size

A number of patients had symptoms suggestive of hypoglycemia Late in the morning there was a feeling of exhaustion anxiety increased perspiration The blood sugar (fasting after food and at intervals after sugar ingestion) was observed in ten individuals The fasting blood sugar showed levels of 33 to 70 mg per 100 cc using a method in which most normal individuals showed from 80 to 110 mg per 100 cc Isolated observations on other patients in the group showed levels of 80 to 95 mg per 100 cc After ingestion of a meal or a measured amount of glucose there was but slight increase in the height of the glucose curve (increased tolerance) and the fall was slow although 3 individuals showed a lower level than the fasting level between three and five hours after the ingestion of the glucose

The serum sodium potassium and chlorine was within normal limits in these patients None of the patients showed unusual pigmentation

In 12 of the patients low basal metabolic percentage had led their physicians to prescribe thyroid without therapeutic advantage however as the abnormal fatigue persisted

The only feature of the urine which was present in most of the individuals was a low specific gravity of individual specimens taken at random during the morning or afternoon Values from 1 001 to 1 008 were common and values higher than 1 010 were unusual in this group

In 3 patients of the group being studied although the onset was typical of acute infectious mononucleosis material from lymph nodes had been obtained by biopsy The sections were variously interpreted by different observers and x ray therapy was given over all the glandular areas by the patients doctors These patients later

CHRONIC INFECTIOUS MONONUCLEOSIS

By RAPHAEL ISAACS M A M D

IN A GROUP of 206 patients who had infectious mononucleosis 53 had some symptoms which persisted for from three months to at least four years or longer The syndrome included ease of fatigue exhaustion aching of the legs weakness depression afternoon elevation of temperature (99 8 to 101 F) moderate splenomegaly low blood pressure low blood sugar often low specific gravity of the urine and the presence of infectious mononucleosis cells in the blood

The 53 patients with the chronic symptoms included 22 males and 31 females The ages ranged from 8 months to 60 years There were 5 younger than 19 years 20–29 years 12 30–39 years 16 40–49 years 15 50–60 years 5 The 8 month old child showed unusual diarrhea (dysentery) since birth and examination of the blood showed infectious mononucleosis cells The mother had acute infectious mononucleosis during pregnancy one month before the birth of the child Thirteen of the patients had the symptoms for from 3 to 6 months 15 for 7–12 months 3 for 17–18 months 11 for one year 8 from two and one half to four years and 3 for at least six years Some of the patients could give the exact date of the start of the symptoms others within a month or so Five gave the duration as several months several years many years

These patients had been sent in for study with possible diagnoses of undulant fever tuberculosis Addison s disease Hodgkin s disease Rocky Mountain spotted fever lymphosarcoma hypothyroidism menopausal syndrome subacute bacterial endocarditis neurasthenia and syphilis

All of the group showed infectious mononucleosis cells in the blood The red blood cell and leukocyte counts and hemoglobin content were within normal limits The infectious mononucleosis cells were of the mature type with deeply basophilic cytoplasm staining the peculiar blue characteristic of these cells The nuclei showed the streaky chromatin with fenestrations and were often indented Occasionally one or more of the large forms found in the acute type were noted The cells constituted 1 to 7 per cent of the total leukocytes rarely higher These cells had been grouped with the lymphocytes or monocytes by uncritical technicians

In no case of the chronic group was the sheep cell (heterophile) agglutination titer above 1 64 Five patients showed persistent positive Kahn tests characterized as general biologic reaction for one to six years

The presenting symptom was always weakness or ease of fatigue The patients said that their legs were weak and ached The fatigue was usually present on arising in the morning but occasionally developed late in the morning or during the afternoon Some had symptoms suggestive of hypoglycemia Others had mental depression nervousness ease of perspiration and dizzy or giddy spells on arising

The fatigue appeared out of proportion to the physical data The usual findings

Three of the group developed characteristics of lymphoblastoma and two showed the features of Banti s congestive splenomegaly

The symptoms responded to treatment with a preparation of adrenal cortical extract

The syndrome is apparently not uncommon and the intense prolonged debility together with the marked improvement after therapy with adrenal cortical extract makes its recognition of great practical importance

had a recurrence of the glandular enlargements and one was diagnosed as lympho sarcoma one reticulum cell sarcoma and one Hodgkin s disease They continued to show clear cut infectious mononucleosis cells in their blood The lymphosar coma patient received intensive x ray irradiation from several doctors as well as nitrogen mustard until he died of emaciation The results make one wonder if lymph nodes injured or made more susceptible by infectious mononucleosis may be made to show malignant characteristics after x ray therapy

In another group 2 patients showed progressive enlargement of the spleen and were later classed as Banti s disease It is possible that some congestive spleno megalies may arise in this way

In the differential diagnosis undulant fever presents the most difficult problem The various tests for degrees of immunity are not diagnostic of the disease and a positive blood culture is not easily obtainable in the chronic form The presence of infectious mononucleosis cells in the blood is a differential point although an individual could have had both diseases When the Kahn test was positive in these patients it was of the general biologic type

Treatment

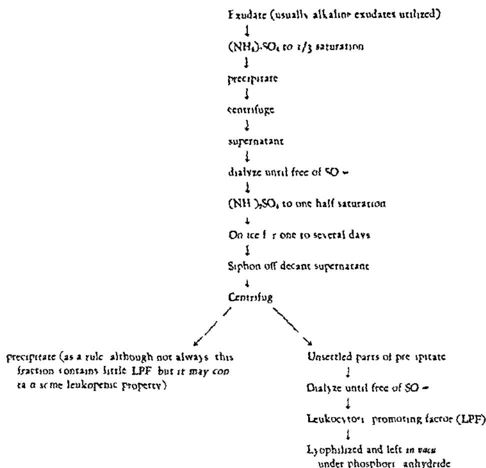
Many therapeutic agents were tested without success These included caffeine amphetamine sulfate (benzedrine and dexedrine) strychnine thiamine atropine multiple vitamin mixtures thyroid ephedrine and special diets These substances produced no lasting effect and often accentuated the nervousness The most promis ing medicine appeared to be a preparation of adrenal cortical extract (cortalex) This was given in doses of 2 tablets (made from aqueous extract of 10 grams of adrenal gland) on arising in the morning There was but little subjective improve ment during the first week but a definite feeling of well being developed during the second week and was quite definite during the third week After this the medi cation was discontinued and the improvement usually continued In a few patients it was necessary to increase the dose or resume it after its discontinuance Associ ated with the subjective improvement there was a decrease in the size of the spleen The changes in the blood pressure were slight

The fact that the symptomatology is somewhat suggestive of adrenal insuffi ciency of the Addison s disease type and that administration of adrenal cortical extract by mouth relieved the patients after the symptoms had persisted for long periods suggests a possible mechanism for the fatigue during the chronic stage There are apparently no data on the appearance of the adrenal cortex in this con dition and whatever damage is present must be reversible if adrenal insufficiency is the cause of the symptomatology

Summary and Conclusions

A group of patients is described in whom ease of fatigue fever splenomegaly low blood pressure low blood sugar low specific gravity of the urine and the presence of infectious mononucleosis cells in the blood persisted for from three months to longer than four years after the initial attack

denaturation has occurred. If the material is then centrifuged the now insoluble part of the precipitate may even induce a leukopenic effect in contrast to the original leukocytosis promoting property which it possessed. This however is not consistently true. At times it has either no activity, or at most a weak activity. On the other hand if the supernatant phase of the insoluble aged LPF is injected



into the blood stream of a normal dog considerable activity is obtained. The supernatant fraction induces a rise in the number of circulating leukocytes. It appears as if aging of the LPF causes a spontaneous denaturation into a relatively inactive and insoluble part. It splits off the active principle in the form of soluble component. The data of several such experiments appear in table I. It is clear that when 10 to 20 milligrams of aged LPF (3–6 months old) is treated with about 10 cc of saline, stirred and centrifuged, the supernatant part yields considerable activity when injected into dogs. There is an increase of about 64 per cent in the number of circulating leukocytes (table 1). The evidence indicates that the active

Determination on three samples of LPF has yielded a recovery on the average of 12.8 milligrams of LPF per cc of exudate.

THE ACTIVE PRINCIPLE IN THE LEUKOCYTOSIS PROMOTING FACTOR OF EXUDATES

By Valy Menkin M D

THE earlier studies of the writer have demonstrated that the leukocytosis accompanying many inflammatory processes is referable to the liberation at the site of inflammation of a factor closely associated with the pseudoglobulin fraction of exudates [1] [3] The factor per se offers a reasonable explanation for the mechanism of leukocytosis with inflammation This factor not only induces a discharge of immature leukocytes into the circulating blood,[1] but it also is capable of producing a hyperplasia of granulocytes and of megakaryocytes in the bone marrow [4] The factor (abbreviated as the LPF) is active on human beings thus suggesting possible clinical application [5] It has been found to be active on guinea pigs This animal may well serve as a convenient assay animal [6] In as yet unpublished studies it has been found that the LPF reinforces the leukocytosis caused by an already existing inflammation This may be significant in the usage of the material in clinical cases with inflammatory processes It is quite conceivable that the factor may be utilized as an adjunct to the antibiotics

The leukocytosis promoting factor has always been recovered in the form of a pseudoglobulin Recent studies in association with Dr G Cooper and Mr M L Dillon at Duke University suggest that the LPF seems to be distributed primarily between the α_1 and α globulins of exudates In the present communication evidence is furnished which suggests that the active group in the pseudoglobulin molecule of exudates is a relative simple polypeptide

Experimental

The leukocytosis promoting factor (LPF) utilized in the following observations has been obtained from pleural exudates in dogs previously injected with 1 5 cc of turpentine as described in the past The scheme of extraction can be briefly restated by referring to the diagram on p 940

The material when freshly obtained appears as a fluffy white powder which is extremely soluble in an aqueous medium It is active in dogs inducing a discharge of immature white cells into the circulation

After several months a curious change occurs in the material It seems to lose its solubility becoming quite insoluble in physiologic saline and at the same time the material loses its biologic activity It now has either no or little activity in causing a rise in the number of circulating leukocytes It seems as if a spontaneous

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The aged samples of leukocytosis promoting factor used in this study were for the most part prepared at Duke University School of Medicine Aided also in part by a grant from the Bristol Meyers Company of New York

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state were treated with crystalline trypsin in amounts varying from a mere pinch of the enzyme to 2 milligrams The length of incubation with the LPF was also variable lasting from about one hour to over twelve hours The treated LPF failed to be inactivated by tryptic digestion The observations are assembled in table 2 It is quite clear that the addition of trypsin has failed to inactivate the factor Following such digestion the injection of the treated material still caused a rise of 111 5 per cent in the number of circulating leukocytes (table 2) The course of an experiment is graphically shown in figure 2 Trypsin is known to hydrolyze complex proteins and also the products of peptic digestion to the peptide stage [8]

TABLE 2 —*Effect of Tryptic Digestion on the Activity of the Leukocytosis Promoting Factor*

Dog No	Material injected	Basal no of white blood cells	Maximum no of white blood cells within 2-6 hours following injection of treated LPF with enzyme
		c mm	c mm
11 T	13 cc LPF + pinch crystalline trypsin	12 100	24 600
12 T	18 cc LPF incubated overnight with trypsin	7 225	11 300
16 T	20 cc LPF incubated with trypsin 2 hours	12 275	25 900
9 T	18 cc LPF incubated with trypsin 2 hours	10 200	30 900
17 T	17 cc LPF incubated 2 hours with 2 mgm trypsin	6 300	8 950
20-T	10 cc LPF incubated 1 hour and 5 minutes with 1 mgm crystalline trypsin	10 900	24 100
9 T	10 cc LPF incubated 1 hour and 25 minutes with approximately 1 mgm crystalline trypsin	10 750	21 [illegible]50
Average		9 9[illegible]4	21 0[illegible]1

Percentage increase in leukocyte level = 111 5%

On the basis of the foregoing evidence it is conceivable that the leukocytosis promoting factor is not a protein at all and therefore it remains intact when subjected to the influence of a proteolytic enzyme

Yet when the newly obtained and active leukocytosis promoting factor is heated for 30-35 minutes at 100 C the whole molecule appears to be denatured and the LPF is likewise inactivated The results of these experiments are summarized in table 3 When the LPF is exposed to such temperature the material is completely inactivated The LPF now yields a rise of 6 per cent in the number of circulating leukocytes in contrast to its original activity of 104 per cent (table 3) The course of such an experiment is illustrated in figure 3

These observations simply indicate that the LPF is thermolabile but neverthe

principle is liberated *in toto* as a soluble component from the now insoluble and aged sample of leukocytosis promoting factor The course of one such experiment is shown in figure 1

TABLE 1 —*Effect of a Soluble Fraction Derived from Aged LPF (3–6 months old) on the Leukocyte Level*

Dog No	Amount of original LPF from which soluble fraction derived	Basal no of white blood cells	Maximum no of white blood cells within 3 6 hours following administration of material
	mg	*cu mm*	*cu mm*
3 T	10	18 850	30 250
3 T	14 5	19 000	46 125
8 D	14	11 000	27 175
5 T	13 5	16 975	24 050
6 T	14	19 000	22 250
8 T	11 5	16 950	27 300
8 T	17	18 700	20 100
9 T	10	16 000	25 700
10 T	20	9 800	16 850
11 T	20	14 700	24 450
Average		16 097	26 425*

Percentage increase in leukocyte level = 64 2%

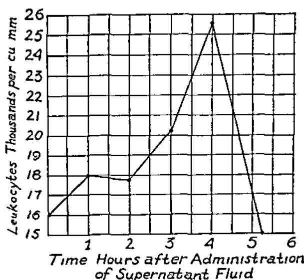


FIG 1 EFFECT OF SUPERNATANT FRACTION FROM AN OLD SAMPLE OF LPF ON THE LEUKOCYTE LEVEL
Dog 9 T Supernatant from 10 milligrams of 5 months old LPF employed

In view of this observation it became of interest to determine whether a proteolytic enzyme would inactivate the leukocytosis promoting factor when freshly recovered as a soluble pseudoglobulin from exudates The LPF was extracted from exudates of dogs as described above Various quantities of the factor in the fluid

ration of the active supernatant material even over a steam bath fails to inactivate the principle These experiments are collected together in table 4 When such brittle dried material obtained by evaporation over a steam bath is heated for 30-40 minutes at 100 C the active principle fails to be inactivated (table 4) The active supernatant material when evaporated to dryness forms brittle flakes which

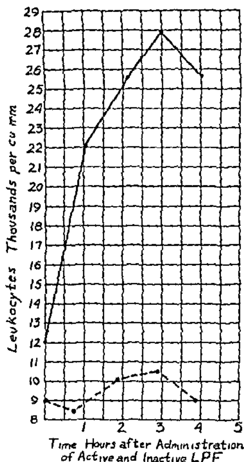


FIG 3 THE INACTIVATION OF THE LPF BY HEAT

Solid line indicates Dog 33 T 25 milligrams of LPF administered Broken line indicate Dog 30-T 25 milligram of LPF inactivated by heating at 100 C for 35 minutes

are insoluble in an aqueous medium Heating again to 100 C such insoluble material fails to decrease its potency Its injection in dogs induces a rise of 114 7 per cent in the level of circulating leukocytes This observation definitely indicates that the LPF can be recovered from aged exudates as a highly thermostable substance Experiments of this sort appear in figure 4 Such observations would preclude the interpretation that the leukocytosis promoting factor is a thermolabile substance adsorbed to the pseudoglobulin molecule *

* The insoluble brittle flakes obtained when evaporating the active supernatant to dryness on a steam bath are biuret and ninhydrin positive

less that it may be merely associated with the pseudoglobulin molecule without being an actual part of it

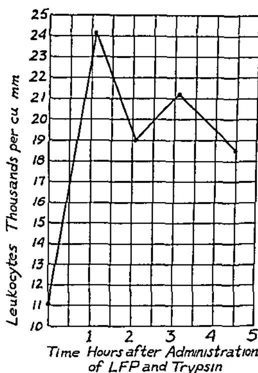


Fig 2 The Failure of Trypsin to Inactivate the LPF

Dog 20-T 10 cc of LPF + 1 milligram of trypsin incubated for 1 hour and 5 minutes before administering to the animal

Table 3 —*Effect of Heat (at 100 C for 30–35 minutes) on the Activity of the Leukocytosis Promoting Factor*

Experiment No	Amount of LPF used	Basal white cell count per cu mm with unheated LPF	Maximum white cell count 3–6 hours following administration of unheated LPF	Basal white count in experiment with heated LPF	Maximum white cell count 4–6 hours following administration of heated LPF
	mg	*mm*	*c mm*	*mm*	*mm*
1	20	8 500	17 400	9 375	9 550
2	19	9 425	19 500	350	8 350
3	17	10 575	16 550	8 825	8 400
4	25	11 800	27 950	9 425	10 600
5	23	11 000	23 250	13 275	14 150
Average		10 260	20 930	9 650	10 210

Percentage increase in leukocyte level with unheated LPF = 104%
Percentage increase in leukocyte level with heated LPF = 6%

Such an interpretation is however not consistent with subsequent facts When now the active supernatant or soluble fraction obtained from aged LPF is evaporated to dryness on a steam bath its activity remains essentially intact Evapo

Polypeptides are known to be highly thermostable.[9] For this reason the amino acid nitrogen before and after hydrolysis was determined on several samples of the active supernatant phase from an aged sample of LPF. These observations are as yet preliminary, but the measurements from such samples indicate in each case a rise in the amino acid nitrogen following acid hydrolysis. The actual figures obtained on such samples before and after hydrolysis are listed in table 5. These

TABLE 5.—*The Amino Nitrogen Content in the Active Principle of the LPF before and after Hydrolysis*

	Before hydrolysis	After hydrolysis
	mg./cc.	mg./cc.
Colorimetric method (modified O. Folin method[1])	0.09	0.2
	0.15	0.37
	0.16	0.51
	0.22	1.00
Average	0.155	0.520
Copper method (method of A. A. Albanese and V. Irby[11])	0.009	0.066
	0.009	0.053
	0.009	0.095
	0.026	0.106
Average	0.013	0.080

observations suggest very strongly that the active principle is a relatively simple polypeptide.

Discussion

The foregoing observations are consistent with the interpretation that the active principle in exudates, which reasonably explains the mechanism of leukocytosis with inflammation, is a relatively simple polypeptide.*

Besides the theoretic significance of this fact there is a possibility that the above observations may have practical application. The canine leukocytosis promoting factor has been shown to be effective in human beings.[5] The active factor has, however, been shown not to be too stable with time. A spontaneous denaturation occurs whereby the material loses its initial solubility. The present observations indicate that in spite of these changes the LPF is split off as a soluble and thermostable component. Its biologic activity remains undamaged. In this way it is conceivable that the leukocytosis promoting factor of exudates can be preserved for long intervals in spite of age. These facts definitely suggest further practical use of this factor.

Conclusions

The leukocytosis promoting factor of exudates appears to be a relatively simple polypeptide attached to the pseudoglobulin of exudates when extracted from the latter.

* The active principle is primarily nondiffusible from the active supernatant fraction of an aged sample of LPF. This would tend to indicate that the factor is somewhat larger than an amino acid.

TABLE 4 —*Effect of the Soluble Fraction Derived from Aged LPF When Evaporated to Dryness on Steam Bath and also When That Dried Fraction is Boiled for 30–40 Minutes*

Dog No	Amount of dried supernatant material derived from aged LPF injected into heart	Basal white cell count	Maximum white cell count following administration of either evaporated material derived from aged LPF or following boiling of such evaporated dried material
	mg	*c mm*	*c mm*
9 T		7 625	17 500
11 T		11 050	24 200
16 T	10	9 350	20 650
29 T	20	12 950	18 400
Average		10 244	20 188*
9 T†	20	13 500	20 350
11 T†	23	11 575	22 100
39 T†	28	14 750	43 050
Average		13 275	28 500‡

* Percentage increase in leukocyte level = 97 1%

† The evaporated material to dryness has in addition been subjected to boiling for 30–40 minutes

‡ Percentage increase in leukocyte level = 114 7%

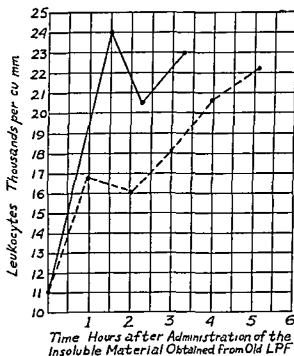


FIG 4 THE ACTIVITY OF THE EVAPORATED SUPERNATANT FRACTION OF OLD LPF

Solid line indicates Dog 11 T Suspension of supernatant fraction from old sample of LPF evaporated to dryness and suspended as insoluble material Broken line indicates Dog 11 T the insoluble material was obtained by evaporating to dryness the supernatant fraction of an old sample of LPF This was then heated for 30 minutes at 100 C The procedure essentially failed to inactivate the material

VARIATION ON THE FORM AND STRUCTURE OF THE NUCLEUS IN THE VARIOUS TYPES OF PLURISEGMENTED NEUTROPHILS

By José Oria, M.D.† and Sakae Yoneda, M.D.

THE HYPERSEGMENTED neutrophil in pernicious anemia is the best known of the various cells with nuclear hypersegmentation as observed in numerous pathologic conditions. Cooke,[2] Naegeli,[16] Kiyono,[14] Rohr,[23] Ponder,[22] Schulten[25] and others described the hypersegmented neutrophil of pernicious anemia and of various serious infectious diseases. Ponder's "polycytes" and Pittaluga's "pleokaryocytes" have been recognized. There are still vague points as to the origin of the plurisegmented neutrophil. Formerly, this hypersegmentation was thought to be a simple process of nuclear ripening. Pittaluga, however, considered the presence of "pleokaryocytes" as a serious toxic sign of nuclear cytoplasmic changes. Naegeli believed that hypersegmentation was a biologic condition of increased regenerative activity and therefore not a sign of senescence. The marked nuclear *polymorphism, according to Naegeli's viewpoint, should not be interpreted as a* simple passive process but as a reflex of the particular biochemical activity. According to Pittaluga, this process is due to an increased proteolytic function in the neutrophils.

Piney[21] referred to the existence in pernicious anemia of large sized polymorphonuclear leukocytes with a hypersegmented nucleus. Cooke[2] pointed out the macropolycytes with multilobed nucleus, counting up to twelve nuclear pieces. Cooke divided these cells into three subtypes, the first as observed in infections, the other two as observed in pernicious anemia. Heck and Watkins[11] stated that in pernicious anemia there may exist an increased segmentation of the nucleus and emphasized the importance of the "finer structure of neutrophil," such as elongation of the nuclear skein and connecting strands, thinning of the lobes and slight condensation of the chromatin. Rohr[23] showed that the nucleus was also inclined to polymorphism and hypersegmentation even during the myelocyte and metamelocyte phases. Dameshek and Valentine[3] stated that the giant metamyelocytes of pernicious anemia presented structural alterations, an increase in the absolute and relative size and a bizarre shape of the nucleus, and that these cells became hyperlobulated, accounting for the hypersegmented neutrophils in the peripheral blood rather than due to an excessive maturation as is ordinarily stated.

Jones[13] referred to the slight inclination of the myeloblast to polymorphism as being in an early change and added that the cells described by Tempka and Braun as "grossere pathologische stabkernige Neutrocyten" can become macropolycytes II and III of Cooke or "pernicious anemia neutrophils" of the peripheral blood as described by Downey. He stated that the hyperpolymorphilic and hypersegmented neutrophils of the peripheral blood of pernicious anemia are completely different from the normal bone marrow and that they are not the result of the functional

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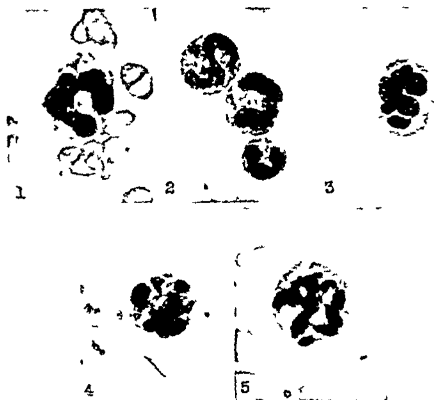
This has been found by a study of samples of leukocytosis promoting factor which have become insoluble by aging Nevertheless the active material has been found to be liberated as a water soluble component which is highly thermostable

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segment by themselves after some time We shall point out how, under certain conditions the artificial hypersegmentation in neutrophils becomes more intensive

In pernicious anemia we have frequently observed two different types of pleokaryocytes one in which the nuclear structures are still young with a very asymmetric and irregular disposition of the lobuli (figs 4 and 5) the other with a



Figs 1 5 —Five instances of the evolution of the Tempka Braun cell to the macroplurisegmented neutrophil Bone marrow Leishman stain Leitz obj 1 1 or 8X)

1 Typical neutrophilic Tempka Braun cell with characteristic nucleus Biermer's anemia 2. The same in a more advanced stage 3 Naegeli paramyeloblastic myelosis plurisegmented cell with 2-lobed nucleus resulting from asymmetric division 4 Biermer's anemia macroplurisegmented cell of the same origin as 1 and 2. 5 The same as 4

more condensed pyknotic nucleus like those of the normal polymorphonuclear neutrophils (fig 6

Thus in our observations of pernicious anemia among the characteristic disturbances of the red cells there is the one of an altered evolution of the granulocytes chiefly of the neutrophils whose anomalous maturation in the nucleus becomes characteristic of this type of anemia Among the neutrophils there are those which present an early segmentation of the nucleus as compared with the insufficient cytoplasmatic maturation with or without a small reduction in volume

alteration of the normal neutrophil as the septic or toxic process He found precocious nuclear polymorphism in the leukoblast promyelocyte or myelocyte phases

Fallon[7] referred to the large neutrophil in pernicious anemia which can contain up to fourteen lobes thin linked by fine strands of chromatin larger and uniform sized granules in the cytoplasm The lobes with the sparser chromatin usually arranged in the peripheral region of the cells

Sturgis and Isaacs [9] recognized Cooke and Hills macropolycytes and mentioned the hypersegmented neutrophils in Biermer s disease Ponder [4] reviewed the polycyte and the propolycyte discussion

Ferrata and Storti[9] stated that the giant hypersegmented originate from the large promyelocyte and myelocyte with more or less distinct nuclear lobes These passed almost directly from the promyelocyte phase to the giant hypersegmented and afterwards to the mature phase The hypersegmented white cells were also observed in the blood of several Mammalia (guinea pig wombat)

The pleokaryocytes can be artificially obtained Bucciardi and Lenzi[1] injected urease in guinea pig and observed a great increase in the polymorphonuclear white cells (up to 65 per cent)

Mezquita Vich and Vich (*ap* Kiyono[14]) worked with oxalated blood in physiologic salt solution kept in an incubator They obtained maximum effects after twelve hours The destruction of these elements began sixty hours later They were successful in repeating this phenomenon by mixing normal venous blood with plasma from patients with tuberculosis cancer and pernicious anemia Ponder [4] was able to produce the polycyte in vitro with venous heparinized blood

Oria [0] pointed out the lability of the several elements in oxalated and centrifuged blood The mononuclear elements especially those which were unripe also underwent a nuclear deformation Nearly always there were radiating or symmetric pictures The author believed in physico chemical change of the nuclear cytoplasmic equilibrium caused by oxalation which is increased by centrifugation

Mendonca[17] obtained hypersegmentation of the nucleus of the neutrophil with liquoid (heparin)

Discussion

In this report we shall study the origin of the hypersegmented neutrophils and some connected facts A few scattered references have been made on the pleokaryocytes that originate from certain promyelocytes and myelocytes with disproportional growth especially observed in the marrow of patients with Biermer s disease (Tempka Braun cells)

Tempka and Braun described the giant myelocytes with delicate chromatin the asynchronism of the nucleus and the cytoplasm with the progressive increase of the karyo cytoplasmatic ratio

Attention should be directed to the eventual difference existing between the septic pleokaryocytes and those we are about to describe which originate from the Tempka Braun cells We shall discuss the greater nuclear lability of the polycyte in vitro using oxalated blood because in this medium the white cell nuclei

can be larger than normal and oxyphilic but can also be present in the cytoplasm coarse or fine azurophilic granules in varying numbers according to the stage in which the cells are (figs 4 and 5)

The third type of Cooke s macropolycyte is the cell which also derives from giant cells but which is more developed and more mature

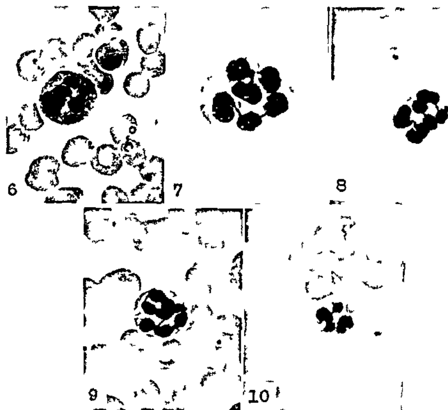
The Tempka Braun type of hypersegmented cells is therefore different from those with equivalent lobulation observed in normal conditions or rather in the septic process in general

Thus as would seem from the above all the authors recognized one type of characteristic pleokaryocyte of pernicious anemia originating from cells described by Tempka and Braun although not all of them agreed as to the origin of the giant or monstrous myelocytes and metamyelocytes Some such as Rohr Dameshek and Valentine Jones Heilmeyer Ferrata and Storti Varela to mention only the more precise assert with conviction the possible origin of hyper segmented characteristic of pernicious anemia from these elements We con firm this including the other hemopathies as well as in chronic leukemic myelo sis Nevertheless few have explained the arrangement of the nuclear chromatin of these hypersegmented nuclei which conserve the characteristics of the undifferenti ated cell during its first phases of evolution Only Jones points out the origin through the myeloblast stage (according to Naegeli) which corresponds to Ferrata s hemocytoblast or the free reticular cell The nuclear characteristics of the undif ferentiated reticular cell can be conserved by Tempka and Braun s cells Thus the hypersegmented forms which derive from them keep the same characteristics of the immature nucleus as the original elements There was only the maturation in the nuclear polymorphism and part of the cytoplasm that is the rest of the cytoplasm can keep specific granulations of the promyelocyte on the other hand the nuclear structure keeps the principal characteristics of the primitive element We not only can demonstrate the leptochromatic nuclear structure but also the nucleoli

According to what can be seen in the normal bone marrow the hyaline hemocy toblasts as described by Pappenheim Ferrata Maximow et al are very rare in normal conditions Only in certain leukemic affections do these cells appear as a backward granular maturation of the undifferentiated reticular cell Most fre quently in these normal conditions one finds them arising directly and imme diately from the reticular cell without an intermediate agranular cell (figs 11 12 13 and 14) The hyaline immature cells of the normal bone marrow are very rare and are hardly ever the real Ferrata s hemocytoblast or Naegeli s myeloblast There is a true cytoplasmic structure of the myeloblastic type (of Ferrata) or promyelocytic (of Naegeli Jones and others) in the free reticular cell *We do not* imply that the giant granuloblast that gives origin to the abnormal Tempka Braun cell in pernicious anemia and leukemia and consequently to the hyperseg mented cells from them originates from a primitive cell of the Ferrata type cell The element described by Ferrata in leukemia with the name of hemohistioblast is a pathologic stem cell (not an artefact as some authors like Naegeli Jones and others believe) The undifferentiated reticular cell that can eventually give origin to the Tempka Braun cell can also give origin to the pathologic hemohistioblast of Ferrata especially in chronic leukemic myelosis These latter do not develop

These cells have a greatly increased nuclear size in a very early stage it is a band form

The karyo cytoplasmatic ratio is therefore increased they are giant cells The cytoplasm *remains more or less basophilic There is no correspondence between the* maturation of the nucleus and of the cytoplasm in these immature neutrophils the early segmentation of the nucleus is not proportional to the evolutive stage of the cytoplasm The cells reveal the nuclear polymorphism in an early stage making it possible also to observe the cytoplasmic vacuolation on and the nuclear perforation



Figs 6–10—Five instances of the evolution of different types of pleokaryocyte originating from habitual neutrophilic cells with more condensed nucleus

6 7 8 Sepsis typical plurisegmented cell with symmetric lobulation of nucleus 9 Leukemic myelosis medium sized plurisegmented cell with pyknotic nucleus 10 Sepsis microplurisegmented cell with pyknotic nucleus

Jones says This whole process reminds one of the granulocytic development which sometimes proceeds from Rieder cells in the leukemias These cells have been described by Tempka and Braun (1932)

Such elements are subject to an exaggerated segmentation leading to the formation of characteristic hypersegmented cells that were described by Cooke Heck and Watkins Dameshek and Valentine Jones Ponder Fallon Schulten Heilmeyer Ferrata and Storti Varela et al The chromatin is delicate young not corresponding to that *of cells with a normal amount of nucleic acid* The granulations

Naturally these references are made with a view to the marrow where the hypersegmented forms derived from Tempka Braun cells show more leptochromatic characteristics than those observed in the circulating blood of course in the periphery there is a certain degree of nuclear maturation that permits one to find in pernicious anemia and l ukemic myelosis hypersegmented cells with a nuclear network that are more pachychromatic and have a more mature aspect

There are cas~s of a ute leukemic myelosis in which the reticular cells of the marrow immediately assume nuclear segmentation simulating monocytoid ele ments (atypical hemocytoblasts and myeloblasts or better Naegeli s paramyeloblasts) The nucleus is immature and although tending to be deformed possesses nucleoli and delicate chromatin network

The nuclear polymorphism of these leukemic reticular cells might lead in certain types of myelosis to the Tempka Braun cell type we have noticed this fact where there was a tendency to paramyeloblastosis (fig 3)

Besides the Tempka Braun cell type there is however the possibility of finding the common hypersegmented cell in leukemias with pyknotic and irregular lobuli as we shall describe for other conditions (fig 9)

We were able to observe in a case of chronic leukemic myelosis in an acute phase the hypersegmented neutrophils originating from cells of the Tempka Braun type where more precocious polymorphism was verified in the pormyelocyte and myelocyte stages We observed vacuoles in the cytoplasm polymorphism of the nucleus of the immature neutrophils as Jones and others described in pernicious anemia The cytoplasm presented alterations which appeared to have a growth slower than that of the nucl us The specific granulations of the hyperpolymorphilic and hypersegmented forms were coarser than normally (fig 15)

As morphologic characteristics it is observed that the leukemic or pernicious segmented cell derived from the Tempka Braun type possesses generally highly irregular and asymmetric lobuli elongated band form and uneven the same happening to the pleokaryocytes which are derived from them and which present a delicate nuclear structure and basophilic cytoplasm The nuclear lobuli are elon gated or rounded more often they do not present both aspects in the same cell In the cytoplasm of the more immature cells we observe the azurophilic granules that can be very fine In the peripheral blood the specific granules of the cytoplasm of the more developed hypersegmented cells are generally larger than normal and of a more or less uniform size (figs 1 2 3 4 and 5)

The asymmetry may easily be explained these cells come from others whose nuclei are subject to an exaggerated and irregular growth in the form of a band that turns on itself in complicated ways sometimes ring shaped and in an arbi trary disposition which accounts for the subsequent marked segmentation One might say that this irregularity is conditioned by the degree of youth of the chromatin for the symmetric segmentation of the neutrophil nucleus a certain maturation level of the chemical constituents of the nucleus is necessary especially the nucleo-proteins as observed in the hypersegmented cells derived from normal myelocytes

This theoretic view point is not wholly destitute of a practical basis as was shown by the experiments of Ponder and MacLeod (1938) These authors using injections

into the Tempka Braun cell because they are abortive elements with a certain character different not only in the pachychromatic nuclear structure but also in the partial specific maturation of cytoplasm The observers who recognized the so called leukemic histiocytes or Ferrata s cells know perfectly well that they are characteristic elements of chronic leukemic myelosis and that they have nothing to do with the hemohistioblast a synonym of the undifferentiated reticular cell

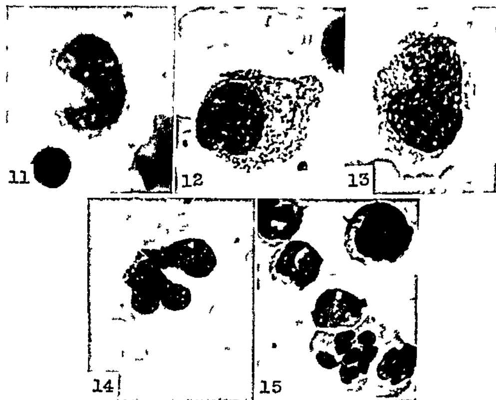


FIG 11 —One stem-cell of atypical progenies of Tempka Braun granular cell Note the characteristic morphology (shape and size) the expressive nucleoli

FIG 12 —First phase of the evolution of the free reticular cell with immediate granular maturation two types of azurophilic granulation First stage of the cellular specimens observed as follows in figures 13 14 and 15 (Leishman stain L itz obj 1/12 oc 10×)

FIG 13 —Evolution of the Tempka Braun type the giant metamyelocyte of Tempka Braun corresponds to the same immature element of the first with the myeloblastic character of the cytoplasmic and nuclear structure with only a beginning of nuclear segmentation (Same coloration and magnification as 12)

FIG 14 —Fourth phase of the same anterior elements (11 12 13 pernicious anemia Sternal marrow) (Same coloration and magnification as 12 and 13)

FIG 15 —Hypersegmented from Tempka Braun cell in peripheral blood of chronic leukemic myelosis

In a recent publication Ferrata and Storti[9] do not identify the primitive reticular cell with the leukemic cell which has only a few general resemblances Therefore in this work when we refer to Ferrata s hemohistioblast we wish to designate the cells that can under special circumstances produce giant elements with a lepto chromatic nucleus (not the pachychromatic type of the leukemic Ferrata cell) re sponsible for hypersegmented forms derived from the giant Tempka Braun cells

form and dimensions some are elongated others rounded still others button shaped in their principal piece or sometimes the rounded lobuli are placed in line etc At any time a pyknotic structure and tendency towards elongation of the lobules is present Sometimes pleokaryocytes derived from segmented neutrophils possess more or less rounded pieces like those seen in septic processes The nuclear disposition however is generally more or less irregular The cytoplasm is acido philic and contains a full quota of specific granules

In septic processes the aspect is quite different There is a third type of hyper

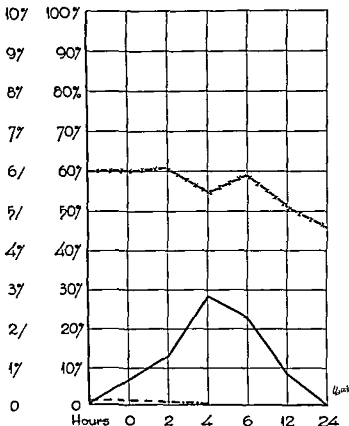


FIG 17—(Same as fig 16) Blood from normal adult male There is a p rcentage increase in the pluri s gmented cells at the exp nse of normal band and segmented cells The maximum reached after 4 hours is in keep ng with all other observations made und r the same conditions (Sam symbols as fig 16)

segmented neutrophil In these septic pleokaryocytes one may well observe the symmetry of the nuclear arrangement that is the nuclear symmetry of the Pitta luga type of pleokaryocyte or of the polycytes of Ponder which is not found in the two first types Besides there is a tendency to the rounding of the lobuli (figs 7 and 8)

In sepsis the cell is activated beyond its working capacity and owing to this increased metabolism the process of amitotic nuclear division is intensified within certain limits and according to the degree of the septic process there is no separa

of nucleic acid, obtained an increase in the shift to the right of the normal neutrophils in the peripheral blood of rabbits

Although in a reduced proportion we have sometimes observed cells similar to those of the Tempka Braun type with hyperpolymorphilic and hypersegmented nucleus but of smaller size To those hypersegmented cells we might give the name of micropleokaryocytes of the Tempka Braun type

In the peripheral blood of pernicious anemia the normal neutrophils of the

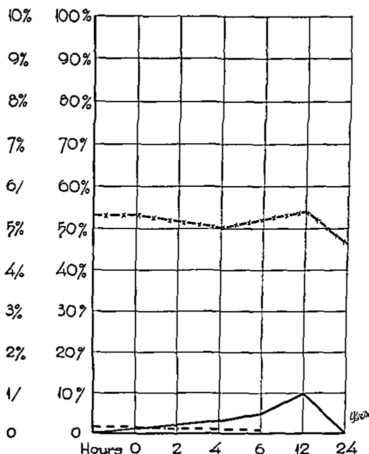


Fig 16—Comparative study on the alteration of the neutrophils from recently collected blood (Hour 0) and from oxalated blood stored 24 hours In every case there is a production of plurisegmented cells in oxalate blood variable in time and intensity according to the case

Blood from normal adult male While the number of stab cells tends to disappear the segmented increase proportionately reaching a maximum at the end of 12 hours These cells disappear at the end of the maximum limit of conservation

—X—X— segmented — — — band ——— multilobulated neutrophil

granulocytic series may also tend to produce hypersegmented cells as in the septic processes with no passage through the above mentioned phase Fallon says that besides the so called pernicious anemia neutrophils toxic neutrophils can be found Thus a second type of hypersegmented cells is obtained in which the nuclear pieces are almost always unequal and take preferably an elongated shape The lobuli are irregular and more or less asymmetrically disposed with variable

form and dimensions some are elongated, others rounded still others button shaped in their principal piece or sometimes the rounded lobuli are placed in line etc At any time a pyknotic structure and tendency towards elongation of the lobules is present Sometimes pleokaryocytes derived from segmented neutrophils possess more or less rounded pieces like those seen in septic processes The nuclear disposition however is generally more or less irregular The cytoplasm is acido philic and contains a full quota of specific granules

In septic processes the aspect is quite different There is a third type of hyper

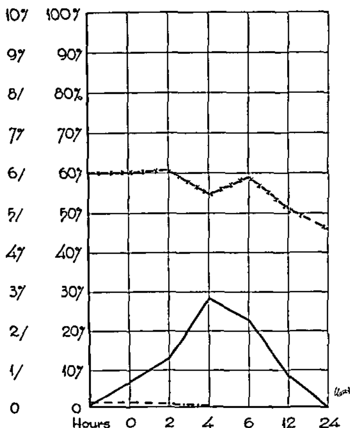


Fig 17 —(Same as fig 16) Blood from normal adult male There is a percentage increase in the pluri segmented cells at the expense of normal band and segmented cells The maximum reached after 4 hours is in keeping with all other observations made under the same conditions (Same symbols as fig 16)

segmented neutrophil In these septic pleokaryocytes one may well observe the symmetry of the nuclear arrangement that is the nuclear symmetry of the Pittaluga type of pleokaryocyte or of the polycytes of Ponder which is not found in the two first types Besides there is a tendency to the rounding of the lobuli (figs 7 and 8)

In sepsis the cell is activated beyond its working capacity and owing to this increased metabolism the process of amitotic nuclear division is intensified within certain limits and according to the degree of the septic process there is no separa

tion of the nuclear segments which remain united by delicate chromatin filaments The result is an increase in hypersegmented neutrophils with a shift to the right of the Arneth index The more intense the work of the cell the more typical are the direct nuclear divisions and more pronounced the amitotic like constrictions This fits very well into the queston of the symmetric nuclear constrictions described by one of us (J O) in the leukemoid processes (1935) One of us confirmed what Di Guglielmo considers the direct division of the nuclear mass of the neutrophils

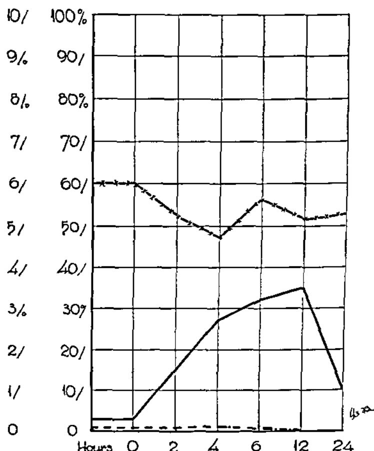


Fig 18 —(Same as fig 16) Pulmonary tuberculosis Very activ production of pleokaryocytes already ob rved in the first f v hours (increased fragility) reaching maximum at th end of 12 hours following the o cillation of th s gmented neutrophils nd not holly disapp aring at th end of 24 hour (Sam symbol as fig 16)

without corresponding division of the cytoplasm The cytoplasm is full of some what coarse specific granulations and may sometimes show degenerative vacuoles

One may occasionally observe eptic pleokaryocytes with some elongated segments with pyknotic structure like the second type as pleokaryocytes found in pernicious anemia However the disposition of the lobule is generally more regular and symmetric In several cases it is possible to find a type of hyperseg mented cell of small volume very pyknotic nuclear structure this is a degenerative type which coincides with that described by Pittaluga as karyoschisis The

nuclear lobuli are frequently superimposed on each other In severe cachetic processes, we also have seen numerous pleokaryocytes including forms with karyoschisis which consist *in the elimination of pyknotic nuclear particles in the* terminal degenerative phase

Consequently, the study that we have made is related to the lability of the segmented neutrophils in these severe conditions (tuberculosis cancer etc) We have tested the increase in fragility of these cells by chemical mechanical or physico

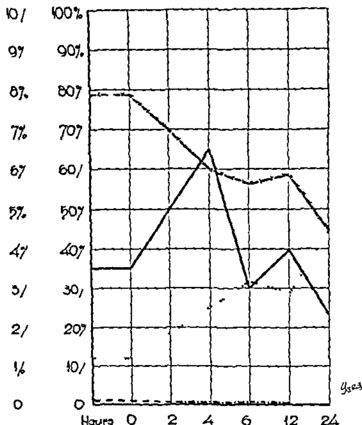


Fig 19 – (Same as fig 16) Accentuated fragility of neutrophils in stored blood Sharp rise in 4 hours of the existing plurisegmented cells with percentage fall in the normal band and segmented cells The per cent increase of neutrophils on the twelfth hour depends on the destruction of lymphocytes the lymphocyte rise on the twenty fourth hour indicates destruction of neutrophils due to increased fragility (Same symbols as fig 16 lymphocytes)

chemical means Submitting the blood of patients with the above mentioned conditions *to the action of oxalate* with or without centrifugation we attempted to investigate the increase in pleokaryocytes which are usually found *in the* untreated blood of such patients

Making differential counts in smears prepared with blood from the finger and blood kept with oxalate at different intervals we observed that the more segmented and developed neutrophils and also the younger forms are generally more

labile than those of normal individuals After some time there is an increase in the number of artificial pleokaryocytes as was observed by Mezquita Vich and Vich with a final reduction owing to the destruction of the cells

Figs 16 17 18 and 19 of unselected observations demonstrate the greater resistence of the neutrophil nucleus in oxalated blood of normal individuals In pulmonary processes and in sepsis this resistance is considerably reduced paralleling the pre existence in vivo and neoformation in vitro of hypersegmented neutrophils

In sick subjects due to the greater lability of the young and segmented neutrophils there is an increase in the percentage of lymphocytes in the oxalated blood There is a much greater variability in differential counts than in normal individuals

The chemical test of preserved blood with or without centrifugation which is positive in normal or abnormal cases becomes strongly positive in the blood of patients severely affected

Finally one must recall here the appearance of hypersegmented cells outside of the human species They may be found as characteristics of certain species even in apparently normal conditions One of us (J O) has already called attention to this fact (1928) In Brazilian *Xenarthra* we have further observed the appearance of pyknotic pleokaryocytes and the same phenomenon of karyoschisis The observation was also made in *Didelphis aurita*

SUMMARY

The hypersegmented neutrophilic leukocytes which originate from the Tempka Braun cells are of a larger size their nuclear segments are also asymmetrically arranged but on the other hand the latter are of variable shape and size and the chromatin network is a delicate and immature one This is a regenerative or at least *not a degenerative* pattern

Asynchronism exists between the maturation of the nucleus and the cytoplasm in the type of hypersegmented neutrophilic leukocyte which occurs in pernicious anemia (second type) This presents many elongated lobes which are asymmetrically arranged in the cytoplasm and have a condensed chromatic network

The type of hypersegmented neutrophilic leukocyte which occurs in *sepsis* presents a normal size and a circular outline its nuclear lobes have a degenerative pyknotic chromatin structure and are symmetrically arranged in the cytoplasm

The hypersegmented neutrophilic leukocytes should not be studied in stored blood which causes artificial changes

The nuclear fragility of these leukocytes may be studied in every case which shows great alterations of the granulocytes

In oxalated blood hypersegmented leukocytes develop frequently and rapidly

The authors also give a description of the hypersegmented leukocytes of some wild and laboratory animals

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BONE MARROW NUTRITION IN RELATION TO THE PHAGOCYTIC ACTIVITY OF BLOOD GRANULOCYTES

By Clarence A. Mills, M.D., Ph.D.

PHAGOCYTOSIS has long been recognized as the body's first line of defense against invasion by micro-organisms, with the wandering blood granulocyte playing a particularly important role. Humoral factors which influence the phagocytic activity of these cells have received much attention as basically important elements in the immunity mechanism. Little work has been devoted, however, to the nutritional background of these cells at the time of their production in the bone marrow. Knowledge recently acquired indicates that it is just as important for these fighting units to be "well born" as it is for the whole individual; that granulocytes produced in the bone marrow during periods of malnutrition or vitamin deficiency remain poorly functioning units throughout their lifetime, while those arising from properly nourished marrow tissue emerge with and maintain full phagocytic activity.

In the large group of respiratory infections and in numerous incidental exposures such as those of burns and wounds, our chief defense against bacterial invasion lies in the basic activity of the phagocytes, unreinforced by the humoral mechanism which may become an important stimulant to phagocytosis only after two to three weeks' exposure. It is therefore essential that we be aware of the conditions promoting or hindering the output of fully active phagocytic granulocytes from the bone marrow.

Development of a quick and relatively simple technic for measuring phagocytic activity of blood granulocytes[1] opened the way for an intensive study of the physiology of these cells in experimental animals kept on synthetic diets.

Methods and Results

A review of the literature concerning phagocytosis and consideration of the various possible technics convinced us that our best chance to ascertain quantitative differences in phagocytic activity lay in the study of blood leukocytes in vitro. The following technic was used.

Under light ether anesthesia, 0.5 ml. of blood is withdrawn from the rat's heart into a syringe previously rinsed in heparinized salt solution. This blood is immediately transferred to a paraffined tube of 10 mm. inside diameter and mixed with 0.5 ml. of salt solution containing ⅛ mg. of heparin. To this heparinized blood is added 0.2 ml. of a standard bacterial suspension (see below); air is washed from the tube by a stream of O_2–CO_2 mixture (95%–5%) to maintain a normal blood gas level; the tube is stoppered with a paraffined cork and inverted twice for thorough mixing. It is then placed in a 38° C. water bath and agitated with a lateral motion (560 reversals of direction per minute) for four minutes. A sample is then removed with a small paraffined pipet and a smear is made which is dried and treated with Wright's stain. Four blood samples are usually run together as a group. Careful watch was kept at all times of the temperature of the water bath, for changes greater than 1° C. produced distinct alterations in phagocytic activity.

Polymorphonuclear neutrophiles of rat blood tend to clump much more readily than do those of man, especially after active ingestion of bacteria has taken place. With the technic just described, however, clumping is rarely observed. The second difficulty—still not completely solved—was a tendency of the

From the Department of Experimental Medicine, University of Cincinnati, Cincinnati.

phagocytic cells to spread unevenly in the smear They often are found concentrated in the last portion of the smear and may be missed unless a very small drop of blood is used so that it is all on the slide for examination The most active cells with largest numbers of ingested bacteria seem most prone to collect in the tail end of the smear

In estimating the number of ingested bacteria after four minutes of shaking counts were made on the first 40 unruptured and unclumped polymorphonuclear neutrophiles seen on the smear if there were less than 4 rats to the group counts were made on 50 cells from each blood sample In a few test cases the ingested bacteria were counted in 100 cells but the accuracy of the mean count was no greater than with 40 or 50 cells

The organism used in these first basic studies on vitamin deficiency was Micrococcus candidus It was chosen because its nonpathogenicity greatly facilitated the running of large numbers of phagocytic tests while its fairly large size simplified the ingestion counting The organism was grown on tryptose agar slants with transfer every twenty four hours The culture used was a saline suspension of a 24 hour growth with a turbidity carefully standardized for each day s work in an Evelyn electrophotometer

Various shaking times and speeds were used in our early work with the plotting of ingestion curves however for the organism and speed of shaking chosen by us a single four minute reading seemed to give as much information as did a whole series carried out over a fifteen minute period Longer shaking was needed when a culture of Type I pneumococcus was used since ingestion seemed to take place more slowly A coagulose positive staphylococcus on the other hand was found to be ingested more readily than the micrococcus What was desired was shaking sufficiently prolonged to give only partial filling of the phagocytic cells in the normal control tube so that deviations toward more or less active ingestion could be measured In our shaking only lateral to and fro motion was used with the agitation insufficient to break the blood surface or cause bubble formation

Vitamin deficiency studies in rats Sprague Dawley white male rats were used in all the in vitro phagocytosis studies of vitamin deficiency except those with vitamin C in which guinea pigs served as test subjects Weanling rats were placed 2 to the cage in groups of 4 in the cold and hot rooms and given the following diet mixture in glass jars ad lib

Sucrose	76 Gm /100 Gm diet mixture
Casein (vitamin free)	18 Gm /100 Gm diet mixture
Corn oil	2 Gm /100 Gm diet mixture
Salts	4 Gm /100 Gm diet mixture
Haliver oil	1 2 ml /1000 Gm diet mixture
Thiamine chloride cold room	1 mg /1000 Gm diet mixture
hot room	2 mg /1000 Gm diet mixture
Riboflavin	4 mg /1000 Gm diet mixture
Pyridoxine	4 mg /1000 Gm diet mixture
Calcium pantothenate	6 mg /1000 Gm diet mixture
Nicotinic acid	25 mg / 000 Gm diet mixture
Inositol	1 Gm /1000 Gm diet mixture
p Aminobenzoic acid	0 3 Gm /1000 Gm diet mixture
Choline cold room	0 75 Gm /1000 Gm diet mixture
hot room	5 0 Gm /1000 Gm diet mixture

This diet with the thiamin and choline increase for the hot room rats usually gives optimal growth in both heat and cold and serves as an excellent standard diet for rat deficiency studies Graded reductions in any one of the vitamins result in corresponding diminution of growth rate At least three weeks are needed for complete adaptation of animals to the hot and cold environments but a somewhat longer time is required to bring out the full growth retarding effects of vitamin

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unquestionably mathematical significance in the rat data here set forth With hot room groups 1 and 4 of the pyridoxine series for instance the difference is 7 67 ±0 55 the difference being fourteen times its own probable error and with only an infinitesimal likelihood of ever occurring by chance along Even the difference of 4 11 ±0 34 (cold room groups 1 and 3 of the thiamin series) is twelve times its own probable error

The testing of each series of rats was done in the course of a single half day using one dilution of the bacterial culture for all tubes It is conceivable that many of the organisms might have died during the course of the two hours or so elapsing between the running of the first and last groups of the series thus accounting for the rising ingestion rate However no significant difference was found when the culture suspension was tested on normal bloods after standing for intervals up to five hours after the dilution was made nor did the use of a heat killed culture alter the rate of ingestion Furthermore the order of testing was always to finish the groups of one room and then go on to those of the series in the other room It is thus not possible to account for any of the observed differences on the basis of change in the culture The samples of heparinized blood stood about fifteen minutes on the average before being run but even five hours of standing at room or water bath temperature had no effect on white cell activity

We have made no use of continuous observations of phagocytic activity of single cells since different neutrophiles of the same animal may show rather marked variations in activity The statistical approach seemed more appropriate and has been used throughout In registering ingestion counts any cell was considered full when it contained 30 or more bacteria Beyond this point accurate counting became impossible because of the crowding

In the entire absence of any vitamin phagocytic counts often rose from their usual low level when the condition of the deficient animal became critical It is to be noted also that our highest ingestion counts were always obtained in animals receiving the diet richest in the vitamin concerned Studies are now in progress to see whether this relationship would continue with still higher concentrations of vitamin in the diet

In addition to the studies shown in table 1 preliminary tests have indicated that lack of vitamins A and D (combined) produces a similar reduction in phagocytic activity Inositol and p-aminobenzoic acid have so far been found to be without effect

Effects of vitamin C deficiency on phagocytosis in guinea pig blood Four groups (4 to the group) of young guinea pigs were placed on a basal diet consisting of wheat bran (45 per cent) rolled oats (25 per cent) and dried skimmed milk (30 per cent) Three drops of haliver oil were given weekly to supply vitamins A and D One group in the hot room and one in the cold were given plenty of leafy vegetables in addition to the basal diet while the second group in each room got no vitamin C After three weeks the weight of those getting no vitamin C showed no gain as contrasted of an average gain of about 60 grams per pig in the control groups Phagocytic tests at the end of the 3 week period gave the ingestion findings as shown in table 2 using the same technic as in the rat studies

deficiency Table 1 shows the differences in body weight and white cell phagocytic activity of rats adapted to various graded vitamin deficiencies

TABLE 1—*Rat White Cell Phagocytosis in Graded Vitamin Deficiencies*

Group	Amount of vitamin per kilo of diet	At 68 F		At 90-91 F and 60-70% R H	
		Aver body weight at end of period	Number of bacteria ingested in 4 min	Aver body weight at end of period	Number of bacteria ingested in 4 m
		Thiamin deficient rats tested after 4 weeks on diets			
		gm		gm	
1	0 6 mg	89	3 40 ±0 21	63	2 63 ±0 16
2	1 0 mg	146	3 66 ±0 22	87	5 27 ±0 23
3	2 0 mg	154	7 51 ±0 27	124	7 40 ±0 24
		Riboflavin deficient rats tested after 7 weeks on diet*			
1	0 0 mg	71	5 76 ±0 27	81	3 81 ±0 21
2	1 0 mg	165	8 13 ±0 25	181	4 51 ±0 24
3	2 0 mg	182	9 37 ±0 28	216	7 54 ±0 25
4	4 0 mg	196	12 00 ±0 30	227	11 48 ±0 51
		Pyridoxine deficient rats tested after 7 weeks on diets*			
1	0 5 mg	129	4 95 ±0 40	167	7 18 ±0 27
2	1 0 mg	184	6 82 ±0 36	192	9 23 ±0 29
3	2 0 mg	204	8 60 ±0 29	196	10 83 ±0 32
4	4 0 mg	217	13 56 ±0 42	202	14 13 ±0 41
		Pantothenic acid deficient rats tested after 7 weeks on diets			
1	0 5 mg	76	2 43 ±0 16	100	2 55 ±0 21
2	1 0 mg	114	3 63 ±0 21	89	3 78 ±0 22
3	3 0 mg	151	4 58 ±0 22	161	6 83 ±0 21
4	6 0 mg	198	5 89 ±0 27	149	5 92 ±0 27
		Choline deficient rats tested after 6 weeks on diets			
1	0 0 Gm			147	3 08 ±0 25
2	0 2 Gm	180	2 47 ±0 15		
3	0 4 Gm	178	4 88 ±0 27		
4	0 75 Gm	19-	6 53 ±0 23		
5	1 5 Gm	178	6 72 ±0 33		
6	3 0 Gm			170	5 40 ±0 26
7	5 0 Gm			172	8 11 ±0 32

The riboflavin and pyridoxine series were run earlier than the others using a somewhat heavier culture suspension this accounts for the greater number of organisms ingested

In every series of rats deficiency of any one vitamin sufficient to retard growth also caused a reduction in phagocytic activity of the white blood cells This reduction was most marked in the riboflavin and pyridoxine series in which the rats had been kept on the deficient diets for seven weeks before being tested In thiamin deficiency of four weeks duration there was a marked reduction in ingestion rate

Group differences of 2 or more in the number of bacteria ingested per cell are of

Here we see best phagocytosis and best growth taking place at 18 per cent dietary protein in the cold room rats with slight growth impairment and marked reduction in phagocytic activity as protein intake is increased above this level In the hot room on the other hand both growth and phagocytic activity continue to improve with rising protein intake even up to the 36 per cent level While the differences in phagocytosis between contiguous groups of rats are not mathematically significant those between the high and low groups of each room are highly so The difference between groups 1 and 3 of the cold room (2.98 ±0.37) is eight times its own probable error and would occur by chance only once in 14 700 000 times Similarly the difference of 3.56 ±0.39 for hot room groups 1 and 5 is nine times its own probable error Just why phagocytic activity should decline with the higher protein intakes in the cold must be left for future explanation

TABLE 4

	Protein in diet	Bacteria per cell	Aver wt after [illegible] weeks
	%		gm
At 68 F	6	3.54 ±0.22	70
	12	3.[illegible]6 ±0.24	156
	18	6.52 ±0.30	206
	24	4.66 ±0.21	205
	36	3.28 ±0.21	201
At 90–91 F and 60–70% relative humidity	6	3.76 ±0.23	51
	12	4.78 ±0.24	100
	18	5.62 ±0.23	157
	24	5.39 ±0.26	195
	36	7.32 ±0.32	203

From these observations it seems evident that protein intake is fully as important as proper vitamin supply in maintaining optimal phagocytic activity The casein used here as the total protein supply is poor in cystine but even when 0.2 per cent cystine is added to all diets there still is evidence of a higher protein requirement in the heat than in the cold

Time lag in phagocytic response to changes in nutritive state Preliminary observations had indicated that full vitamin-deficiency effects on phagocytosis would be in evidence after four weeks on the deficient diets It seemed desirable however to have more definite information on the time relationships involved

Weanling white rats (Sprague Dawley males) were placed in tropical warmth (90 to 91 F and 60 to 70 per cent relative humidity) and kept on synthetic diets for eight months before being used for the study The rats in one group received the optimal diet for tropical warmth described previously while those in the other group received a diet moderately deficient in protein and all the B vitamins (table 5)

While this low vitamin rat diet would appear to be only mildly deficient it was about as low as would be tolerated by 8 month old rats Further reduction of

A second series of guinea pigs were kept in the rooms on the basal diet for four weeks Graded amounts of ascorbic acid were given daily by pipet these amounts being 0 5 mg 1 5 mg and 3 0 mg per day per pig All those in the cold room receiving no ascorbic acid died near the end of the fourth week before they had been tested for phagocytic activity Those of the corresponding hot room group died during the fifth week Tests on those remaining alive at the end of the fourth week gave the results as shown in table 3

The guinea pig bloods were highly unsatisfactory to work with because of troublesome clumping and fragmentation of the phagocytic cells Even with all due reservations as to the accuracy of the counts however there is no doubt of a marked reduction in phagocytic activity in severe vitamin C deficiency

TABLE 2

	Numb r of ba t r a i ge ted per cell
Cold room full diet	18 30 ±0 23
no vitamin C	7 30 ±0 30
Hot room full diet	16 12 ±0 44
no vitamin C	8 20 ±0 28

TABLE 3

A corbic acid	Bacteria pe ell t 68 F	Ba t ria p ll at 90–91 F nd 60–70% rel h m
mg /p g/d y		
0 0		5 02 ±0 38
0 5	7 42 ±0 56	15 53 ±0 69
1 5	11 90 ±0 38	17 86 ±0 79
3 0	12 02 ±0 59	19 27 ±0 81

Protein deficiency studies in rats Increasing emphasis is now being placed on the role of body proteins in resistance to infection and on the maintenance of acquired immunity Cannon s excellent discussion of the subject pictures the loss of protein attached immune bodies as tissue reserves of protein are depleted from any cause (blood loss protein starvation and the malnutrition accompanying vitamin deficiency debilitating disease or old age) No mention has been made however of the part reduced phagocytosis might play in such loss of resistance Hence we decided to study this phase of the subject in conjunction with our work on vitamin deficiency and differences in protein requirement in heat and cold

Using the phagocytic technic and basal diet described in the preceding pages we adjusted the protein and sugar content of the basal diet to give 6 12 18 24 and 36 per cent protein and corresponding reductions in sugar All vitamins were kept at optimal levels with the needed increases in thiamin and choline in the hot room Weanling white rats (males) were placed on these diets in the hot and cold rooms in groups of 4 After five and one half weeks on the diets the rats were bled and phagocytic tests run as described The data obtained are as shown in table 4

alterations were found in activity of the blood phagocytes By the end of the second week a moderate change was observed in phagocytic function this became more marked during the third week and was complete by the end of the fourth week (fig 1) After four weeks the rats that formerly had deficiencies exhibited normal phagocytic activity while those previously normal were now at the low level of full deficiency

With bacterial counts made in 40 phagocytes from each rat (making 160 cells for each mean value recorded in the table) differences in one day s readings greater than 1 5 became statistically significant Naturally the readings of one week can be compared with those of another week only by reference to each week s normal values obtained on the control rats The bacterial suspension used in the third week s test was slightly too dilute while that of the fourth week was distinctly

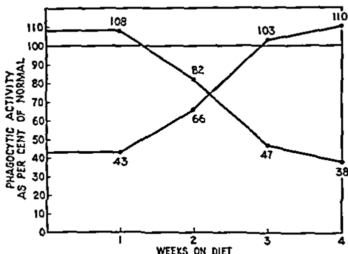


Fig 1 Phagocytosis in rat malnutrition and recovery

heavy This necessitated the calculation of comparative changes on a percentage basis *using the normal controls as 100 per cent in every case Differences of* ±10 per cent are of no definite significance here

One review journal has recently[2] criticised our use of only 40 cells per rat (four rats to each group) as the basis for calculating mean ingestion rates referring to the custom of counting ingestion from 200 to 500 cells in human phagocytic studies To test the stability of our ingestion values we selected two representative groups from our previous report Groups 1 and 3 of the riboflavin series in the cold room In addition to the mean values calculated on counts from 160 cells per group (40 per rat) we recalculated the data on the basis of including only the first 20 10 5 and 2 phagocytes seen on each slide In table 7 are set forth the results of this recalculation and it is clearly indicated that the observed differences in mean ingestion counts maintain their statistical significance when as few as only the first 10 cells per rat are included in the calculation It may thus safely be accepted as

thiamine from 1 2 mg per kilogram down to 1 0 mg per kilogram resulted in typical severe deficiency symptoms and death within four to five weeks Rats of this age kept since weanlings in the heat on the optimal hot room diet also develop fatal thiamine deficiency in about the same time if the dietary thiamine is reduced to 1 0 mg per kilogram

Using the technic described above we measured the phagocytic activity of the blood polymorphonuclear neutrophiles and then placed the rats with deficiencies

TABLE 5 —*Amounts of B vitamins and Casein per Kilo of Diet*

	Optimal diet for tropical warmth	Deficient diet
Thiamine hydrochloride	2 5 mg	1 2 mg
Riboflavin	4 0 mg	1 5 mg
Pyridoxine	4 0 mg	1 5 mg
Calcium pantothenate	6 0 mg	1 5 mg
Nicotinic acid	25 0 mg	10 0 mg
Choline chloride	5 0 Gm	1 0 Gm
Inositol	1 0 Gm	0 4 Gm
p Aminobenzoic acid	0 3 Gm	0 1 Gm
Casein vitamin free	180 Gm	120 Gm

TABLE 6 —*Weekly Changes in Phagocytic Activity*

	Control rats on full diet	Time on new diet	Rats changed from full to deficient diet		Rats changed from deficient to full diet	
	Mean number of bacteria per cell		Mean number of bacteria per cell	% of normal	Mean number of bacteria per cell	% of normal
		wk				
	6 12 ±0 36	1	6 59 ±0 33	108	2 65 ±0 18	43
	6 17 ±0 37	2	5 04 ±0 27	82	4 10 ±0 31	66
	5 37 ±0 33	3	2 51 ±0 20	47	5 51 ±0 28	103
	7 57 ±0 37	4	2 87 ±0 19	38	8 31 ±0 29	110
Wt changes						
First week			−10 gms		+17 gms	
4 weeks	+16 gms		−68 gms		+61 gms	
Initial average wt	340 gms		346 gms		169 gms	

on the optimal diet while changing some of the normal rats to the deficient diet Estimates of phagocytic activity and weight change were made weekly thereafter for each group Four rats in each group were bled from the heart and discarded from the study at the end of each week so that the study would not be complicated by any possible effect on phagocytosis from repeated bleedings

In table 6 are set forth the changes in phagocytic activity and body weight which took place from week to week Even though the dietary shifts in each case were promptly reflected in body weight changes within the first week no corresponding

granulocytes in experimental animals This effect of a faulty diet or of a restoration to normal after a period of malnutrition alters the phagocytic activity of circulating granulocytes only after a lag of two to three weeks thus leading to the conclusion that these cells are susceptible to nutritional faults only during their early formative period in the marrow tissue

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true that counts made on 40 phagocytes per animal with four animals to each group allow an ample margin of stability In human case studies 100 cell counts would provide sufficient stability if the technical details of the method were carefully standardized

TABLE 7

No cells counted		Group 1		Group 3		Group diff in means	Size of diff in means needed for sig mean (P E X 4)
Per rat	Per group	Mean number bacteria ingested per cell	Standard deviation of mean	Mean number bacteria ingested per cell	Standard deviation of mean		
40	160	5 76 ±0 27	5 12 ±0 19	9 37 ±0 28	5 28 ±0 20	3 61 ±0 39	1 56
20	80	5 23 ±0 38	5 03 ±0 27	8 88 ±0 37	4 97 ±0 26	3 65 ±0 53	2 12
10	40	4 55 ±0 52	4 92 ±0 37	8 10 ±0 51	4 77 ±0 36	3 55 ±0 73	2 92
5	20	6 20 ±0 86	5 72 ±0 61	7 90 ±0 85	5 63 ±0 60	1 70 ±1 21	4 84
2	8	6 25 ±1 46	6 12 ±1 03	6 75 ±1 14	4 79 ±0 81	0 50 ±1 85	7 40

Discussion

From the results here presented it would seem that the ability of bone marrow to produce active phagocytes is dependent on the same nutritional requirements needed for optimal body growth Deficiency of any one of the B vitamins (except inositol or p-aminobenzoic acid) or of protein sufficient to retard body growth also interferes with the marrow production of normally active phagocytes

This effect of nutritional deficiency seems to be exerted only upon phagocytes during their early immature period in the marrow tissue for nutritional correction—which at once restores normal growth—fails to bring back normal phagocytic activity to circulating granulocytes except after a lag of between two and three weeks It therefore seems justifiable to conclude that granulocytes already in the circulating blood are not influenced by changes in nutritional status that they are susceptible only during their early formative period This means that improved phagocytosis cannot be expected until two to three weeks after nutritional faults have been corrected

Fewer as well as less active phagocytes are produced in deficiency states the total leukocyte counts in rats dropping from a normal level of around 10 000 per cubic millimeter down to about 4 000 without any marked change in the differential count

Preliminary observations have shown phagocytosis to be 4 to 5 times as active in some human subjects during the summer months than through the winter season Whether this winter decline in activity is related in a causative way to the greater susceptibility to colds and other respiratory infections forms interesting grounds for speculation

Conclusions

Deficiency of any of the B vitamins (except inositol or p-aminobenzoic acid) vitamin C or of protein leads to a reduction in phagocytic activity of the blood

following pathologic involution It was the main problem in the present investigation

That the bulk of the supporting stroma is composed of an epithelial remnant of the embryonic anlage has been shown in detail by Hammar,[3-6] Maximow[1, 2] and Hartmann [7] The epithelium becomes disorganized by the immigration and proliferation of lymphocytes and assumes a superficial resemblance to the reticulum of lymph nodes Hart [8] (1912) Rudberg [9] (1907) Pappenheimer[13] (1910) Hammar [3-6] Ssyssojew[26] (1924) Wituschinski[27] (1926) Marine[28] (1932) claim that this epithelial reticulum can form histiocytes macrophages and giant cells but the conclusion of Schaffer[11] and of Gottesman[16] and Jaffe [17, 18] (1924) that this tissue can also produce genuine lymphocytes is denied by the above authors and most other students of the problem Ssyssojew [26] who studied accidental involution in children concluded that the cells of the epithelial reticulum behave exactly like those of the reticulo-endothelial system and that they should be included with this system in spite of their different origin

The problem is difficult because mesenchymatous tissue becomes mixed with the epithelial portion and it is usually impossible to distinguish the two tissues in sections of the late fetal and adult stages

Fibers interpreted as connective tissue fibers were seen in the thymus by Watney[29] (1882), Bell[30] (1906) Mietens[31] (1909) Pappenheimer[13] (1910) Salkind[32] (1912) Hartmann[7] (1915) Strandberg[33] (1918) and Jolly[21] (1923) Their presence except for those radiating from septa and blood vessels is denied by Badertscher[34] (1915) Strandberg [33] using the Bielschowsky method made an intensive study of the fibers in the human thymus from fetuses newborn and adults up to the age of 54 He found that the black silver staining fibers increased with age They were most abundant in the medulla and the boundary between cortex and medulla They radiated from the septa and from the blood vessels which penetrate the organ and they penetrated the medulla for considerable distance beyond the vessels and septa He could not determine relationship of fibers to cells and could not distinguish between epithelial cells and mesenchymatous reticular cells The article is accompanied by excellent lithographed plates The writer s preparations of rabbit thymus stained with azo carmine show practically identical relationships of the blue staining fibers to parenchyme as those described by Strandberg for the human thymus

Mietens[31] (1909) had observed numerous fibers penetrating the epithelial reticulum of the medulla in human and animal thymus from deep ends of septa and from perivascular tissue The fibers become embedded in this reticulum but they do not contribute to its formation Numerous thin fibrils in the cellular reticulum were thought to be precollagenous while the thicker ones derived from septa and perivascular tissue of the larger vessels and stained blue with Mallory are collagenous

Jolly [21] in 1923 and earlier agreed that the thick fibers are collagenous He could not stain the thin fibrils of the epithelium with Mallory s connective tissue stain but could stain them with iron hematoxylin after prolonged mordanting and so interpreted them as mitochondrial formations similar to those of the epidermis

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CYTOLOGY OF RABBIT THYMUS AND REGENERATION OF ITS THYMOCYTES AFTER IRRADIATION WITH SOME NOTES ON THE HUMAN THYMUS

By HAL DOWNEY, PH D

With the assistance of RUBY ENGSTROM M A , M D

IT IS now generally conceded that the thymocytes are lymphocytes having an embryonic origin in the mesenchyme surrounding the epithelial anlage of the thymus as shown by Maximow,[1, 2] Hammar [3-6] Hartmann [7] and others A few investigators (Fulci,[8] Goldner [9] Dustin [10] Schaffer [11] Stohr,[1] Pappenheimer [13, 14] Winiwarter [15] Gottesman[16] and Jaffe[17, 18]) have derived the free thymus cells from the epithelium believing that they are special parenchymatous elements of the organ and not lymphocytes or they are genuine lymphocytes derived from the epithelium (Schaffer [11] Prenant [19] Gottesman[16] and Jaffe[17, 18]) However investigations of the embryonic development of the thymus especially the detailed studies of Maximow[1, 2] and Hartmann [7] and experiments with radiation transplantation tissue culture etc (Dantschakoff [9] Hammar [3-6] Jolly [1] Popoff[2, 23]) have convinced most investigators that the thymocytes are true small medium and large lymphocytes identical to those of the lymph nodes and that like the latter they are derived from mesenchymatous tissue

As the normal thymus does not have germinal centers and lymph nodules it can be assumed that most of the lymphocytes of the adult organ the majority of which are small lymphocytes are descendants of the large lymphocytes which invade the epithelial anlage from the mesenchyme of the embryo Hartmann[7] (1915) states that the immigration of these large basophilic ameboid lymphocytes begins at about the fifteenth or sixteenth day in the rabbit fetus and that it subsides gradually Transformation of these cells to small lymphocytes begins on the nineteenth fetal day and all intermediate stages in the transformation are present between the nineteenth and twenty fifth days after the epithelial anlage has been completely penetrated by the large cells

Maximow[1, 2] saw many of these first large lymphocytes in mitosis and concluded that the production of small cells was largely by division of the large ones Hartmann[7] could not determine the exact mechanism and seemed to assume that there is direct transformation of large to small cells Many of the latter were in mitosis which she thought proved that they were not all derived from large lymphocytes

To what extent the local stroma can contribute new lymphocytes especially in the adult is somewhat uncertain The question is important from the standpoint of the maintenance of lymphocytes in the normal organ and their regeneration

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From the foregoing statements from some of the literature it can be asserted that the thymus does contain a considerable amount of mesenchymatous tissue mixed with the epithelial stroma and that it is probably this tissue which produces the fibers described by numerous authors From the character of the fibrils and from its activity in the production of histiocytes and lymphocytes (Popoff [28]) it can be concluded that this tissue is similar to the reticulum of lymph nodes, or that it is *an undifferentiated mesenchyme closely associated with the vessels as claimed by* Popoff

The writer was *interested in determining whether the mesenchymatous reticulum* can be distinguished from the epithelium in imprints or smears of the organ and if it can to what extent it contributes to the formation of new lymphocytes in the normal organ and during regeneration following irradiation He was also interested in studying the thymus lymphocytes in imprints an interest which was stimulated by Dr Arthur Kirschbaum who showed him some very immature lymphocytes in imprints of the normal mouse thymus

Pinner[38] (1915) a student of Pappenheimer s seems to be the only one who has made a detailed cytological study of the thymus by the smear method His interest was focused largely on the question of whether the small thymus cells are lympho cytes or epithelial derivatives

The origin of myelocytes and mature granulocytes of the thymus was another problem studied in the present investigation Their presence in the thymus has been described many times but there is no agreement on their origin Some like Hart[23] and Fulci [8] believe that they immigrate from the blood or connective tissue Hart saw scattered eosinophil myelocytes in the human thymus but believed they were the result of special conditions rather than an indication of hematopoiesis He thought the blood was the most likely source Wituschinski[27] (1926) experi menting with the introduction of foreign bodies in the thymus derived them from adventitial cells of the vessels and from hemocytoblasts some of which origi nated from the epithelial stroma

The prevailing theory for the origin of the thymus myelocytes is that they are derived from the lymphocytes of the organ Maximow[1] (1909) described the de velopment of myelocytes from large lymphocytes of the thymus of fetal rabbit and other animals Danchakoff[10] (1916) showed a similar origin in the thymus of chick embryos after implantation of spleen grafts on their allantois

Weidenreich and his student Weill made a detailed study of this problem The main features were published by Weidenreich[39] (1912) and the details by Weill[40] (1913) Their material was taken from full grown rats and from 4 normal human subjects aged 15 17 19 and 37 years The 19 and 37 year olds were executed and the material was placed in the fixing fluid immediately afterwards The other 2 were cases of accidental deaths

The rat thymus was valuable because of the ring shaped nucleus in the hetero phils and eosinophils which facilitated the study of the development of these cells from lymphocytes All transitional stages in the process could be seen The thymus

Investigations of others show that mixed with the epithelial stroma there is a certain amount of mesenchymatous tissue which is undoubtedly responsible for the production of the reticular fibers The problem was studied by Salkind[3] (1912) Mollier[35] (1913) and in great detail by Hartmann[7] (1915) who investigated the histogenesis of the rabbit thymus Hartmann found that mesenchyme penetrates the embryonic anlage with the connective tissue septa and the blood vessels At first it can be distinguished from the epithelium but later this distinction is impossible As capillaries develop throughout the organ she assumed that they must be accompanied by mesenchyme which has the capacity to form new lymphocytes During development of the thymus the narrow connective tissue septa contain numerous lymphoid cells and this tissue becomes a true lymphatic tissue while the tissue *surrounding the lobules remains as an ordinary connective tissue She noted* that the boundary line between the epithelium and the lymphatic tissue was soon obliterated and that the two tissues intermingled a large portion of the septal tissue becoming a part of the organ parenchyme She believed but could not prove that the mesenchymatous reticulum continues to form lymphocytes One of the important conclusions from this work was that the thymus stroma contains much more mesenchymatous tissue than generally had been assumed

Salkind[3] stated that he could see lymphocytes budding from the mesenchymatous reticulum within the thymus of the adult

Tschassownikow[36] found it impossible to distinguish the two tissues except in tissue culture where they separate

Popoff[23] (1928) a student of Maximow s investigated rabbit thymus by the tissue culture method He found that the epithelium formed islands and a border at the edge of the lobules Some of its cells became isolated from the syncytium but they never phagocytized or stored colloidal dye Later the epithelium degenerated and lymphocytes histiocytes or myelocytes were never formed from it Histiocytes were numerous in the earliest cultures They were derived form the connective tissue partitions between the lobules and in the interior of the organ from *undifferentiated mesenchyme cells about the blood vessels Undifferentiated mesenchyme was found everywhere in close association with the blood vessels and it is this tissue which produces histiocytes and lymphocytes in the autotransplanted* thymus and during regeneration following radiation

Emmart[37] (1936) also experimented with tissue cultures of embryo beef and human thymus and of 3–7 week old rats She found that the outgrowth of epithelial reticulum could be distinguished from that of the mesenchymatous reticulum The cells of the latter were smaller than those of the former and their nuclei were different The epithelial reticulum formed sheets of cells in the later cultures In two or three days after subculture there was an outgrowth of epithelioid tissue which differed from the outgrowth of the other two tissues Its narrower bands had a distinct margin composed of fibroblast like cells Later some of the epithelioid tissue broke up into individual round cells This tissue was assumed to be a remnant of the original epithelium which had not been transformed to reticulum No proof was offered for this conclusion

myelocytes in both sections and imprints it was felt that renewed study of this question might be of interest The imprints seemed to be of special importance because they showed the finest details of nuclear structure which is a valuable feature in determining the origin of cells

Materials and Methods

The animals used were 10 newly born rabbits and 2 young normal rabbits age 4 or 5 months One young rabbit aged 4 or 5 months was irradiated with a dosage of 500 r u This animal was killed on the sixth day after irradiation Its thymus was large One old rabbit was given 400 r u and killed eight days after irradiation Its thymus was in an advanced stage of involution and showed epithelial hyperplasia One old normal rabbit was not irradiated

Some human material was also used for imprints The specimens were from 2 full term newborns 2 stillborns who died shortly before birth one 2 year old male one 5½ months fetus and one premature aged 6½ fetal months

Sections and imprints were made from all of these animals and imprints from the human material Sections were stained with hematoxylin and eosin Dominici s eosin-orange G toluidin blue and with Pappenheim s methyl green and pyronin A few were stained with azocarmine for study of the distribution of connective tissue fibers Sections stained with methyl green and pyronin were dehydrated with dioxan as suggested by Miss Helen Harris of this laboratory Retention of stain was much better than with the usual acetone or alcohol dehydration

The imprints were made by touching a cut surface of the organ to a slide without smearing The preparations were dried rapidly by waving them through the air rapid drying being essential for good films All imprints were stained with the May-Grunwald Giemsa combination The Giemsa stain was used in double strength 6 drops of stock solution in 3 cc of distilled water for each slide for five or six minutes The May Grunwald solution was used like Wright s stain one minute in the stock solution and three minutes after the addition of water The diluted stain was poured off and the Giemsa added without rinsing The slides were not covered because it was intended to use only the oil immersion objective Most of the imprints showed some portions that were good for cytological study

Observations (Rabbit)

The irradiated animals were used for the study of regeneration of lymphocytes in the thymus the others for detailed study of the lymphocytes and of cells from the epithelial and mesenchymatous reticulum and for the granulocytes

There was little histologic or cytologic difference between the thymuses of the newborn animals and those from the young adults aged 4 or 5 months In the newborn there were however fewer large lymphocytes and in the imprints more epithelial cells than were seen in the young adults The presence of more epithelial reticular cells in the imprints of the newborn probably was due to the delicate texture of the organ which caused more of the epithelial cells to adhere to the glass

Epithelial and Mesenchymatou Reticulum

In the imprints the mesenchymatous cells usually could be distinguished from those of the epithelium as shown in figures 5 6 and 7 from newly born rabbits Figures 5 and 6 are epithelial cells and figure 7 is a mesenchymatous cell identical to the reticular cells of lymph node imprints The epithelial cells have rather sharp outlines when they are free and their cytoplasm is of a translucent pale blue color when stained with May Giemsa It is not completely homogeneous for some areas

cells also developed to plasma cells The basophils were of the tissue type and were located in the connective tissue only

The thymus from all 4 human subjects contained neutrophil and eosinophil myelocytes Eosinophils were seen in every section and even in the 37 year old subject about 35 per cent of them were myelocytes The nuclei of the myelocytes varied from large leptochromatic to small pachychromatic nuclei similar to those of the small cells *Many myelocytes were in mitosis Transitions between lymphocytes* and plasma cells were seen also in the human thymus and a few plasma mast cells were noted Other mast cells were of the tissue type

The authors concluded that the free round cells of the thymus are genuine lymphocytes of various sizes similar to those of lymph nodes All types of lymphocytes, even the small ones can develop to granulocytes and plasma cells within the thymus and this is a normal process at all ages

The authors did not mention the possibility of the development of granulocytes from mesenchymatous tissue located within the limits of the thymus parenchyme

Pinner[38] made smears from the human thymus of 25 cases ranging in age from newborn to one year and from one 8 week old rabbit Two types of myelocytes were seen One type had large pale nuclei and was identical to the marrow myelocytes the other type had small dark lymphocytic nuclei Both forms occurred in the promyelocyte stages with few granules Pinner assumed that they were derived from lymphocytes but he did not give any of the details

Hartmann[7] saw some myelocytes with nuclear structure similar to that of large lymphocytes in sections of rabbit thymus She derived the granulocytes of the organ from large lymphocytes but was not certain that they were of local origin because she could not find eosinophil myelocytes containing only a few granules and because the neighboring connective tissue contained many granulocytes

Ssyssojew [6] saw extensive myeloid metaplasia during pathologic involution in 15 children who had died of infectious diseases The neutrophil promyelocytes were identical to the large lymphocytes except for the presence of granules in their cytoplasm There was also myeloid metaplasia in the surrounding connective tissue and in nearby vessels which had its beginning with large lymphocytes Neutrophils were the most numerous granulocytes

Popoff[22] (1926) in regenerating stumps remaining after partial extirpation and of transplants noted the formation of lymphocytes of different sizes from the connective tissue which invaded the hypertrophied epithelium with the vessels Some of these lymphocytes developed to myelocytes which were mostly of the darkly nucleated micromyelocytic variety

Fulci[8] (1913) stated that the thymocytes are not lymphocytes because they do not differentiate to plasma cells or granulocytes However the foregoing extracts from the literature indicate that there is good evidence for the origin of granulocytes from these cells and Schaffer [11] Weidenreich[39] and Dantschakoff[10] showed that they can form plasma cells As the material studied by the writer contained many

characteristics of the nuclei of the reticular cells from which they are derived These large cells and the medium sized lymphocytes have more cytoplasm than is seen about the nuclei of the lymphocytes of the thymus and the nodes have many more extremely basophilic cells of all types Extremely basophilic cells are present in the thymus but on account of the narrow rim of cytoplasm they are not very conspicuous The nodes also contain many more immature lymphocytes than are present in the thymus A detailed description of the lymphocytes of the lymph nodes and their regeneration will be found in the paper by Sundberg and Downey[41] (1942)

Small lymphocytes with very dark pachychromatic nuclei are the most numerous They have so little cytoplasm that often it cannot be seen The chromatin blocks are very dense and stain very dark blue which is almost black Slightly less numerous are larger small lymphocytes with about the same nuclear structure but with chromatin less dense and staining violet rather than blue black

Komocki[19] (1930) described and illustrated the dense nuclei of the small lymphocytes of the thymus He believed that the smallest cells have no cytoplasm Policard Dustin and Marcus were quoted as supporting this opinion He believed that the nuclei of the smallest lymphocytes of lymph nodes have a different structure and that they always are surrounded by cytoplasm He agreed with Schridde that the nuclei of the small thymocytes are more loosely constructed than are those from the corresponding cells of the nodes His illustrations of the small thymocyte nuclei are accurate but the figures of lymphocyte nuclei from the lymph of lymph nodes do not in any way resemble the nuclei of lymphocytes of either sections or

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Fig 4 Promyelocyte of mast leukocyte The nucleus is similar to the one of fig 3 Same imprint as figs 1–3

Figs 5 and 6 Epithelial reticulum cells from imprint Note nuclear groove in fig 5 and clumping of chromatin in fig 6 The cytoplasm is characteristic and unlike that of the mesenchymatous cell of fig 7 Same imprint as figs 1 4

Fig 7 Imprint of cell from mesenchymatous reticulum Newborn Compare nucleus and cytoplasm with the epithelial cells of figs 5 and 6 Note characteristic stippled nucleus and azurophilic granules in cytoplasm of fig 7

Figs 8 and 9 From imprint of newborn Fig 8 Lymphoblast showing very diffuse distribution of chromatin In fig 9 there is some clumping of chromatin and some stippling suggesting origin of this cell from mesenchyme

Fig 10 Section young normal adult Development of heterophils from mesenchymatous reticulum under capsule Nucleus of upper granulocyte similar to nucleus of mesenchyme Cytoplasm of mesenchyme cell not shown because it is unstained until granule begin to develop The lower granulocyte shows that chromatin increases as the cytoplasm fills with granules

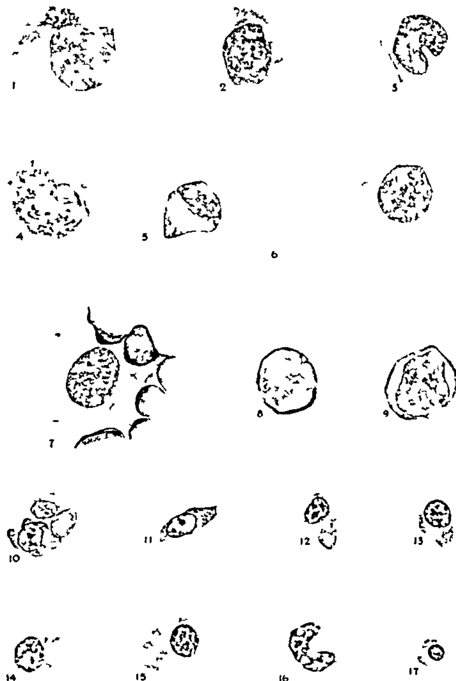
Figs 11 and 12 Section young normal adult Hemocytoblastic myelocytes in septum A slight amount of basophilic cytoplasm is still present

Fig 13 Section young adult Heterophil myelocyte in cortex

Figs 14 and 15 Imprint young adult Lymphocytic heterophil promyelocyte and myelocyte with nuclei of small lymphocytes Compare these nuclei with those of figs 1 2 and 4

Fig 16 Imprint young adult Heterophil metamyelocyte which seems to have developed from a cell like 15

Fig 17 Section Eosinophil myelocyte with nucleus of small lymphocyte This cell was one of a group of 5 similar cells in interlobular connective tissue Eosinophils of this type and transitions between them and the bilobed form occur in the medulla All the eosinophils of the rabbit thymus seem to develop from small lymphocytes Figs 14 and 15 prove that heterophils also may develop from small lymphocytes



All figures are from thymus of rabbit All were painted ith the same magnification Leitz $\frac{1}{12}$ oil immersion objective and ocular 10X Camera lucida outlines The imprints ere stained with May Grun ald Giemsa and the secti ns ith D m n ci s eosin-orange G toluidin blue

Figs 1 2 3 Pseudoeosinophil prom elocyte and metamyelocyte Imprint from 5 day-old rabbit In fig 1 the nucleus shows some tippling of chromatin that is characteristic of cells of the me enchyma tous reticulum like the one of fig 7 suggest ng o gin of granulocytes from this reticulum Such an origin as seen in sections is illustrated in fig 10

The presence of these transitional stages suggests that there is some development of lymphocytes from mesenchymatous reticulum in the thymus of normal animals In the sections of normal animals it was impossible to see this line of development However during regeneration after irradiation of the thymus as seen in sections stained with methyl green and pyronin development of lymphocytes from the fixed tissue seems to be increased This is probably the best stain that can be used for study of this process The transition from fixed cells to free lymphoid cells was noted in several places at the periphery of the lobules near the capsule and in the medulla close to the larger blood vessels Downey and Weidenreich[15] (1912) published descriptions and colored illustrations of the development of lymphocytes from reticulum in sections of lymph nodes stained with methyl green and pyronin The process is similar to what was seen in the irradiated thymus

From the character of the intermediate cells of the imprints it seems clear that the fixed cells involved in this process are not of epithelial origin as some authors have assumed. Hartmann [7] better than any other author has given an explanation of the presence of mesenchymatous tissue in portions of the cortex and in the medulla in the neighborhood of blood vessels The mesenchyme which penetrates the organ in early embryonic stages is undoubtedly the fixed tissue which is capable of forming lymphocytes under certain conditions Hartmann believed this but she was unable to demonstrate it with the methods she used

The presence in the imprints of reticular lymphocytes similar to those of the nodes can be explained by the activity of the mesenchymatous tissue in the production of lymphocytes However this does not account for the immature lymphocytes of the blast type similar to those of lymphatic leukemia Naegeli[18] believed that the lymphoblasts were not blasts but lymphocytes which were about to divide by mitosis or had just completed a division This might be an explanation for the presence of such cells in the thymus but it does not account for their absence in lymph nodes Mitoses were not very numerous in the normal thymus material used in this study Their number was increased during regeneration following irradiation and the blast cells also seemed to be more numerous The lymphoblasts do not seem to be an intermediate stage in the development of lymphocytes from the mesenchymatous reticulum However the thymus is not very favorable material for study of this question because the lymphoblasts are so few in number The development of similar cells from the reticulum has been seen in human lymphatic leukemia by Fineman[16] (1922) and by Stasney and Downey[17] (1935)

GRANULOCYTES

As noted in the introduction many investigators have seen myelocytes in the normal animal and human thymus Some like Hart [4] thought they resulted from special conditions and so were not to be interpreted as a sign of hematopoiesis Others believed they were formed in the connective tissue surrounding the thymus or were brought in by the blood Pappenheimer[13] [14] interpreted the eosinophil myelocytes as specifically differentiated epithelial elements which might have a secretory function Hartmann[7] could not find eosinophil myelocytes containing

imprints of nodes Pappenheimer[13] (1910) also believed that most of the small thymocytes have no cytoplasm that can be detected in smears in which respect they differ from lymphocytes of lymph node smears The thymocytes were thought to be epithelial elements

It is true that most of the small thymocytes have very dark checker board nuclei with sharply demarcated chromatin blocks separated by rather broad pale interspaces Many of these nuclei are suggestive of the type seen in small plasma cells Similar cells occur in lymph nodes of the same animals but they are not as numerous and their chromatin blocks usually are not as darkly stained Naked nuclei similar to those of the thymus also occur in the nodes Lymphocytes that are slightly larger than the smallest ones usually have cytoplasm in both nodes and thymus

The writer concludes that every type of lymphocyte of the thymus can be duplicated in the nodes However certain types predominate in each of these organs as has been shown in the preceding discussion

Next in number in the thymus are medium and small large lymphocytes usually with the nuclear structure of mature lymphocytes Cells corresponding to the largest lymphocytes of the nodes do not occur The cytoplasm of these thymus cells forms a narrow band about the nucleus it is basophilic but usually not as dense and homogeneous as in the nodes It usually has a light area at one side of the nucleus Lymphocytes with abundant cytoplasm are scarce

In sections narrow bodied large lymphocytes are scattered among the small and medium ones of the cortex There are very few large or medium sized ones in the medulla The largest ones with the most abundant cytoplasm tend to collect under the condensed connective tissue at the surface of the lobules Here they approach in size the large lymphocytes of the nodes although the nodes have some lymphocytes larger than any that occur in the thymus Blood vessels of the medulla sometimes are surrounded by a dense collar of small and medium lymphocytes a condition which seems to be more frequent during regeneration after radiation

In the thymus imprints there is a fair number of large lymphocytes with very immature nuclei In some of these cells it is diffusely distributed in the form of a delicate network or fine stippling without any clumping of chromatin One such cell is shown in figure 8 Cells of this type are sufficiently immature to be called lymphoblasts They resemble the lymphocytes of acute leukemia more than they do the immature reticular lymphocytes of the rabbit nodes These blast cells are not numerous Cells like the one of figure 9 in which there is some clumping of chromatin are more numerous The nucleus of this cell has some characteristics suggesting origin of the cell from a mesenchymatous reticular cell with a nucleus similar to that of figure 7 There is some clumping of chromatin but there is also some coarse stippling of the chromatin like that of figure 7 Some transitional forms between 7 and 9 could be found but they were not nearly as numerous as in lymph nodes Illustrations of these transitional stages in lymph nodes are to be found in the papers of Downey and Stasney[4] (1936) and Sundberg and Downey[41] (1942)

blocks separated by clear interspaces and it stained very dark blue (fig 15) or violet as in figure 14 This difference in color probably is due to variations in the degree of spreading of the cells and their nuclei The nucleus may be in the center of the cell but usually it is eccentric (figs 14 15) A cell of this type with a central nucleus as seen in section is shown in figure 17

In the marrow type of promyelocyte the cytoplasm is quite basophilic when the first granules appear In the lymphocytic type the small lymphocytes develop a medium or wide cell body which becomes almost colorless The first granules appear as very small pink specks in this colorless cytoplasm A few of these cells with the nuclei of small lymphocytes (figs 14 15) have a slightly basophilic area with few granules and a colorless area in which most of the granules are concentrated (fig 14) Cells of this type are not very numerous That they complete their development is indicated by cell 16 which seems to be a metamyelocyte of this series Several pseudoeosinophils with this type of nucleus and transitional stages between this and the lobulated nucleus of the mature heterophil were found in the imprints

In the sections most of the heterophil (polymorphonuclear) myelocytes are of the hemocytoblastic type of figures 11–13 Their nuclei are identical to those of the large basophilic lymphocytes of the sections Their nuclear membrane is thick the chromatin blocks are coarse and there is good staining of the karyoplasm Remnants of basophilic cytoplasm are often present (figs 11 12) and in the younger promyelocytes it is obvious that the granules develop in a basophilic cytoplasm A few heterophil myelocytes with dark compact nuclei corresponding to those of figures 14 and 15 of the imprints were also seen in the sections

All transitional stages in their development from large basophilic lymphocytes to mature cells can be found Development of granules does not begin until the lymphocytes have acquired more than the usual amount of basophilic cytoplasm Cells that are in compact groups are often in the same stage of development thus there may be several metamyelocytes in one group and only myelocytes in a neighboring group

The distribution of these cells is quite irregular they are numerous in some lobules particularly the smaller ones that project for some distance into the surrounding connective tissue Other lobules contain none They are most numerous in the outer portion of the cortex close to the capsule but they can also be located in any part of the organ They are either scattered or in compact groups and may be numerous in the medulla in the neighborhood of large blood vessels and connective tissue strands

Another type of pseudoeosinophil myelocyte which usually occurs in groups of 2 or 3 cells immediately under the capsule is seen occasionally Two such cells are shown in figure 10 Their nuclear membranes are thinner and the nuclei are paler than those of the hemocytoblastic type due to the lighter staining of the karyoplasm and the smaller and more diffusely distributed chromatin granules The cytoplasm of the younger promyelocytes with few granules is only slightly basophilic as seen in the upper cell of figure 10 The basophilia increases as the cells acquire more granules (lower cell of fig 10)

only a few granules, so concluded they were developed outside the thymus while the heterophils were of local origin Weidenreich[39] and Weill[40] are among the few who have studied the granulocytes in sufficient detail to trace their origin and transformation to mature leukocytes

In the imprints there are two types of granulocytes in the heterophil series viz , a type similar to those of the bone marrow (figs 1–3) and the lymphocytic type (figs 14–16) described by Weidenreich [39] Weill[40] and Tuve [48] In the youngest promyelocytes of the bone marrow type granules are not basophilic as they often are in the marrow The basophil myelocytes (mast myelocytes) that were seen (fig 4) were of the bone marrow type and were similar to the cells of the heterophil series (polymorphonuclears) except for the color and size of the granules Eosinophil myelocytes and leukocytes were not numerous in the imprints The nuclei of the myelocytes were of the small lymphocyte type as shown in figure 17 which however is from a section Imprinting does not change the morphology of these cells to any great extent

The cells illustrated in figures 1–4 were compared to similar cells in excellent rabbit bone marrow smears loaned by Dr Dorothy Sundberg of this Department The lymphocytic type of myelocyte (figs 14–16) was not seen in the marrow but cells comparable to those of figures 1–3 were numerous However there were some differences in structural details between the two series The heterophil myelocytes *of the thymus (figs 1 2) tend to spread out more and assume more irregular out*lines than is true of the corresponding marrow myelocytes This probably is due to difference in the character of the tissues Owing to the thin spreading of the cytoplasm the granules of the thymus cells appear larger than those of the marrow

In many of the marrow heterophil myelocytes some of the granules are basophilic or of intermediate tints between basophilic and acidophilic In the thymus the granules are always bright red The only basophilic granules seen were in the mast leukocytes and their myelocytes (fig 4) The coarse dark myeloid azure granules of irregular shape and size which are present in many of the marrow promyelocytes were not seen in the thymus imprints

Nuclear structures of the myelocytes from the two tissues were quite similar The nuclear pattern of the earliest promyelocytes is quite diffuse It becomes coarser as the cells mature as seen in figures 1–3 The nucleus of figure 1 shows some chromatin blocks which are not as dense as those of the more mature cells and it also shows some stippling of the chromatin In the marrow some of the promyelocytes have a more leptochromatic myeloblastic type of nucleus than those of the thymus This may be only an apparent difference due to the greater number of myelocytes in the marrow which facilitates the finding of the various developmental stages

The lymphocytic type of heterophil myelocyte seen in the imprints and illustrated in figures 14–16 was not seen in the marrow The granules are identical to those of the marrow type of cell (figs 1–3) but the nuclei are quite different and the cytoplasm never shows more than a trace of basophilia The nuclei are identical to those of small or medium lymphocytes Their chromatin is in dense

blocks separated by clear interspaces and it stained very dark blue (fig 15) or violet as in figure 14 This difference in color probably is due to variations in the degree of spreading of the cells and their nuclei The nucleus may be in the center of the cell but usually it is eccentric (figs 14 15) A cell of this type with a central nucleus as seen in section is shown in figure 17

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These cells develop from the stroma under the capsule This reticular stroma is probably of mesenchymatous origin Its nuclei have slightly thicker membranes more chromatin and absence of nucleoli to distinguish them from the nuclei of the epithelial reticulum One such nucleus is shown on the right of figure 10 The cytoplasm could be seen but was practically colorless so has not been included in the figure The nucleus of the upper myelocyte is identical to this nucleus although somewhat smaller The nucleus of the lower myelocyte has more chromatin and slightly darker karyoplasm, but it is not the lymphocytic type of nucleus of figures 11–13 It is possible that such a cell may eventually resemble the lymphocytic type This point could not be settled because there are so few myelocytes developing from the fixed tissue Some cells similar to the upper one of figure 10 were seen in the medulla where there may be much mesenchymatous tissue It is logical to assume that they also have originated from mesenchymatous reticulum

The rabbit is an animal that has few eosinophil leukocytes in its blood marrow and thymus While the total number in the thymus seems to be small they may be numerous in the medulla of some lobules Their development in rabbit marrow from myeloblasts was described by Ringoen[49] (1921) who showed marked changes in size and staining reaction of the granules as they matured These changes in staining reaction of the granules were not seen in the imprints or sections of the thymus The granules are larger and not as bright red as the pseudoeosinophil granules and they are more refractile They are often spindle shaped in the sections Only a few of the mononuclear eosinophils had cytoplasm that was not completely filled with granules which sometimes were a little smaller than those of the more mature cells

Small and medium lymphocytes with dark checker board nuclei seem to be the progenitors of all the eosinophils which are developed in the thymus and of a few of the marrow eosinophils When the nucleus becomes bilobed the chromatin pattern remains essentially that of the small lymphocyte although there may be some condensation of chromatin to form the cart wheel type of nucleus Tuve[48] and Weill[49] described two types of eosinophil myelocytes in human thymus those with large leptochromatic nuclei and those with the nuclei of small lymphocytes Only the latter type could be found in the rabbit

Mononuclear eosinophils like the one of figure 17 often occur in groups in the interlobular connective tissue and in the septa The cell of figure 17 is one of a group of five similar cells Because of the number of these cells in the group and because similar cells were seen in the imprints one cannot assume that the nucleus is merely a section through one lobe of a bilobed nucleus as was claimed by Schridde[50] for similar cells Single cells of the same type are found occasionally in the medulla and in some portions of the medulla near large vessels and connective tissue strands they and mature eosinophils with bilobed nuclei are quite numerous

Mature mast leukocytes (basophils) with lobulated nuclei and a few of their promyelocytes and myelocytes were seen in the imprints of the thymus They could not be identified in the sections probably because the rabbit mast granules are very soluble in water One of the promyelocytes is illustrated in figure 4 The granules have a violet or purple color with May Giemsa staining and they vary somewhat

in size and shape The nucleus of cell 4 is typical of the other basophil myelocytes that were seen *Its structure is identical to that of the bone marrow myelocytes* whose origin could be traced to the myeloblasts In the marrow smears examined the cells are not spread as thin as the one of figure 4 which probably accounts for the smaller size and darker staining of the granules of the marrow cells Tissue basophils are very scarce in the rabbit and none was seen in the thymus of this animal

The origin of some of the granulocytes of the thymus is not clear and comparison *of granulocytes of sections with those of the imprints is sometimes difficult* The cells of figures 1 to 3 seem to belong to one series If one saw only cells 2 and 3 he would be inclined to believe that they were derived from lymphocytes on account of their nuclear structure Cell 1 has a more immature nucleus with some stippling of the chromatin and some rather pale chromatin blocks which are composed of small chromatin granules A nucleus of this type could have originated from a reticular nucleus like that of figure 7 or from the nuclei of immature lymphocytes (figs 8 9) which in turn may have been derived from the mesenchymatous reticulum Cells 2 and 3 show that the nucleus acquires a coarser structure as the cells mature This corresponds to the maturation of similar cells in the marrow and so does not necessarily mean that the cells have been derived from lymphocytes

In myeloid metaplasia lymphocytes may change the structure of their nuclei to the type that is characteristic of the myeloblast before the cells develop granules This dedifferentiation of the cells was noted especially by Dominici[61] (1921 and earlier) and by Maximow[2] (1923) Dominici[61] studied experimental myeloid metaplasia of the spleen and noted that some of the lymphocytes passed through a myeloid stage during their evolution to the granulocyte while others did not *In the myeloid type of evolution the lymphocytes assume the character of myelo*blasts with pale nuclei and little chromatin In the lymphatic type of evolution lymphocytes of any type acquire granules without any further changes in nucleus and cytoplasm In both cases the cells enlarge before producing granules but in the myeloid type the nucleus enlarges more than the cytoplasm so that the cytoplasm becomes relatively narrow and very basophilic The lymphatic type of evolution is the more common one in the normal animal

Maximow[2] (1923) saw myeloid metaplasia in cultures of rabbit lymph node to which cell free bone marrow extract had been added All of the granulocytes developed from lymphocytes In some instances the lymphocytes acquired large pale nuclei before developing granules in others the lymphocytes were practically *unchanged when they developed their granules These observations are identical* to those of Dominici[61] in the mammalian spleen

The dedifferentiation of lymphocytes which may occur before they develop granules makes it difficult to determine in all cases the origin of cells such as those illustrated in figures 1 and 4 especially in the thymus in which the myeloid transformation is not very active in the normal state

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Figures 14 and 15 illustrate another type of heterophil myelocyte seen especially

well in the imprints Their nuclei have the structure of the small or medium lymphocyte the intermediate stages in their development from lymphocytes were seen and have been described In the sections most of the heterophils like those of figures 11 to 13 are derived from large basophilic lymphocytes It is possible however that cells 12 and 13 of the sections correspond to cells 14 and 15 of an imprint although there seems to be too much condensed chromatin in cell 15 for the nucleus of a large lymphocyte

The heterophils of figure 10 which have originated from the fixed mesenchymatous reticulum have nuclei which suggest that these cells correspond to cells of figures 1 and 2 of the imprints In the imprints the mesenchymatous tissue has nuclei similar to the one of figure 7 in sections they appear as in the right hand nucleus of figure 10

REGENERATION OF LYMPHOCYTES AFTER IRRADIATION

From the character of some of the immature lymphocytes in the normal thymus one would suspect that there was some development of lymphocytes from the mesenchymatous tissue However all the transitional stages could not be traced in either sections or imprints Several fields were located similar to the one shown in figure 10 in which granulocytes were developing from the fixed mesenchymatous tissue but a similar origin for lymphocytes was not seen in the normal animal This proves that regeneration of lymphocytes in the normal thymus is homoplastic although it seems likely that a few are derived from the mesenchymatous portion of the stroma

The picture is very different during the active phase of regeneration following irradiation In this material many clear cut instances were seen of the development of lymphocytes from the mesenchymatous reticulum Sections of thymus fixed in Helly s fluid stained with methyl green and pyronin and dehydrated with dioxan were especially good for this problem

The animal used for detailed study was 4 or 5 months old It was irradiated with a dosage of 500 r u and killed six days later Thanks for this are due Dr Harry W Mixer of our Department of Radiology Only the region of the thymus was irradiated The dosage was probably sufficient to eliminate most of the lymphocytes from the organ

Sections showed that recovery was not complete and that regeneration of lymphocytes was still in active progress Mitoses were numerous Although this was a late stage of regeneration the material was very favorable for study of the local origin of some of the lymphocytes

The imprints from this animal contained many more large lymphocytes with immature nuclei similar to those of figures 8 and 9 than were observed in the normal animal of this same age or in the thymuses of the newborn animals Many of the nuclei were of the blast type a few suggested origin from nuclei of mesenchymatous reticulum The increased number of lymphoblasts might be accounted for by the numerous mitoses

In the sections the boundary between cortex and medulla was not very sharp

and often could not be determined The cortex of most lobules was very narrow and its outer portion was very dense with closely packed lymphocytes many of which were of the large variety Mitoses were especially numerous in this region and it was here that transitional stages between fixed tissue cells and lymphocytes were most numerous The intermediate stages were similar to those of figure 10 except that the lymphocytes did not develop granules Sections of this same material stained with Dominici showed that no new myelocytes were being formed and that those present were degenerating Comparison with normal material leads to the conclusion that exposure to x rays reduces or suppresses the local production of granulocytes

Not all of the fixed tissue of the subcapsular region is of mesenchymatous origin and it is only in a few limited areas that one can see the development of lymphocytes from the fixed tissue the characteristics of which have already been described Islands of epithelial reticulum show no signs of activity and this is also true of the compressed epithelium between the numerous lymphocytes of the peripheral cortex

The lymphocytes originating from the mesenchyme are mostly large and they soon develop very basophilic cytoplasm Their nuclei may still have the structure of the mesenchymatous nuclei and have very little chromatin The cell outline usually is quite irregular but tends to become more rounded as the cytoplasm increases in amount and density

Small and medium lymphocytes which have been formed in the outer portion of the cortex are crowded towards the medulla in dense strands between the radially arranged blood vessels In the outer portions of the medulla of some lobules these strands may spread out to form irregularly shaped masses of densely packed lymphocytes Similar dense groups of small lymphocytes may also occur deep in the medulla and in the cortex They may also form dense collars about some of the blood vessels This distribution of lymphocytes was also noted by Christensen and Griffith[3] during accidental involution and early regeneration in rats

Lymph vessels which usually accompany the larger blood vessels are often packed solidly with small and medium lymphocytes Rudberg [5] thought this meant that during regeneration many of the lymphocytes immigrated through the lymph vessels possibly from neighboring mediastinal lymph nodes which regenerate faster than the thymus Both he and Hart [4] stated that the number of mitoses would not account for the rapid production of lymphocytes Rudberg although he worked with many x rayed animals could not see any evidence for migration of lymphocytes from the lymph vessels to the surrounding thymus tissue and this is also true of the material being described in the present study

The development of lymphocytes from the fixed tissue also occurs in the medulla in regions containing abundant connective tissue large blood vessels and often Hassall s corpuscles Here it is again evident that the epithelial tissue which forms the corpuscl s of Hassall is in no way associated with the production of lymphocytes

The relative number of large lymphocytes is not as great in the medulla as in the

peripheral cortex The lymphocytes developing from the fixed tissue are mostly of medium size and they originate from smaller mesenchymatous cells with smaller nuclei than those of the cortex

The other irradiated rabbit was an old animal in which the thymus was in an advanced stage of involution The region of the thymus was exposed for a dosage of 400 r u and the animal was killed 8 days later

The regeneration of lymphocytes is not as extensive as in the younger animal though the time interval between irradiation and death was two days longer

There is marked hyperplasia of the epithelium which forms wide marginal bands in some places and large islands in others The latter usually extend to the surface but may be central These epithelial marginal bands and islands which form during the involution of the thymus were described in detail by Bienert (1923) [51]

Most of the lymphocytes are concentrated in dense masses which usually have a central location but may be more peripheral and extend to the outer margin of the long narrow organ It is difficult to see any reticulum in these regions Epithelium of the islands and marginal bands forms a dense nucleated mass without cell outlines

There are a few scattered lymphocytes and small groups of them in the epithelium It was impossible to detect any mesenchyme associated with the epithelium which could give rise to lymphocytes Large blood vessels are surrounded by connective tissue Development of lymphocytes from this tissue could not be detected A few lymph vessels are filled with lymphocytes and this may be the chief source of the new lymphocytes as there is no evidence for their formation from local fixed tissue in this animal

Rudberg [5] states that after intense radiation which destroys all the lymphocytes the first ones to reappear enter the central portion of the organ where they proliferate and are later distributed to the cortex Conditions in this animal seem to support Rudberg s conclusion It is possible that in the older animals the mesenchymatous tissue if present is no longer capable of producing lymphocytes More extensive material would be required to settle this point In the young irradiated animal there is good evidence for the active production of lymphocytes in the cortex by mitosis and by heteroplastic development from the mesenchymatous reticulum

Irradiation does not cause myeloid metaplasia of the thymus as does accidental involution from infections as described by Ssyssojew [26] in the thymus of children No myelocytes were seen in the thymus of the younger irradiated animal and only one small group of heterophil myelocytes was located in the older animal Two myelocytes of hematogenous mast cells with large nuclei were seen and tissue mast cells with small lymphocytic nuclei were fairly numerous in the older rabbit A few plasma cells and plasma mast cells were also seen There were no tissue mast cells in the thymus of the younger animal

There were many cells which appeared to be macrophages in the sections of the older irradiated animal They contained flakes granular material which stained pale blue or green with the toluidin blue of Dominici s stain and light pink or

yellow with hematoxylin and eosin The nuclei were eccentric and in general appearance the cells were similar to the macrophages of subcutaneous tissue after colloidal dye injection The included material resembles the endogenous granular substance derived from mitochrondria described and illustrated by Tschassownikow[36] in fixed and free reticular cells of cultures of rabbit thymus He found the epithelial cells of the cultures to be only slightly phagocytic The granular cells of my preparations did not contain intact lymphocytes or their nuclei

Some of the nuclei of the granular cells were of the histiocytic or lymphocytic type but many had nuclei similar to those of the epithelial cells including even the spherical nucleoli and nuclear grooves The nuclear membrane often had many wrinkles Some of the fixed epithelial cells also contained the same granular substance The macrophages (?) therefore seem to be of multiple origin from lymphocytes from mesenchymatous reticulum and from the epithelial reticulum It is very likely however that the technique employed did not permit distinction between true macrophages and epithelial cells Tschassownikow[36] (1927) could not make the distinction in sections of rabbit thymus but when he added lithium carmine to his cultures he observed the migration of dye storing macrophages from the explant The epithelium of his cultures did not store the dye

The epithelial origin of macrophages of the thymus was claimed by many authors among whom may be mentioned Rudberg[35] Pappenheimer[33, 34] Hart[14] Wassen[38] Ssyssojew[36] Wituschinski[37] and Marine[28]

Popoff (1926[?], 1928[?]) never saw any macrophages or dye storing cells developing from the epithelium of cultures and transplants He derived all the carmine cells from embryonic mesenchyme about the blood vessels He did not see an intimate mixing of mesenchymatous and epithelial tissues but believed that the perivascular connective tissue would assume embryonic characters where it came in contact with epithelium This is contrary to Wassen[38] (1915) who did not see macrophages growing out from the perivascular tissue of cultures of frog thymus but did see their development from epithelium The epithelium does not form macrophages or phagocytize degenerating lymphocytes according to Christensen and Griffith[6] who obtained rapid involution in rats fed choline-deficient diets

Tschassownikow[36] from his work with cultures concluded that the reticulum is composed of connective tissue elements and epithelium so intimately blended that they cannot be distinguished except when conditions are unusual as during involution and in cultures when the elements may separate and each differentiate in its own particular direction

Wituschinski[37] (1926) claimed that histiocytes and macrophages which gather about a foreign body introduced in the thymus are derived from adventitial and other connective tissue cells and from cells of the endodermal reticulum He also claimed that hemocytoblasts which resemble large lymphocytes are derived from the endodermal reticulum under these conditions The numerous granulocytes which develop around the foreign body he derived from the hemocytoblasts and adventitial cells of neighboring capillaries He did not believe that lymphocytes could be derived from the epithelial reticulum

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There are a few scattered lymphocytes and small groups of them in the epithelium It was impossible to detect any mesenchyme associated with the epithelium which could give rise to lymphocytes Large blood vessels are surrounded by connective tissue Development of lymphocytes from this tissue could not be detected A few lymph vessels are filled with lymphocytes and this may be the chief source of the new lymphocytes as there is no evidence for their formation from local fixed tissue in this animal

Rudberg [5] states that after intense radiation which destroys all the lymphocytes the first ones to reappear enter the central portion of the organ where they proliferate and are later distributed to the cortex Conditions in this animal seem to support Rudberg s conclusion It is possible that in the older animals the mesenchymatous tissue if present is no longer capable of producing lymphocytes More extensive material would be required to settle this point In the young irradiated animal there is good evidence for the active production of lymphocytes in the cortex by mitosis and by heteroplastic development from the mesenchymatous reticulum

Irradiation does not cause myeloid metaplasia of the thymus as does accidental involution from infections as described by Ssyssojew [6] in the thymus of children No myelocytes were seen in the thymus of the younger irradiated animal and only one small group of heterophil myelocytes was located in the older animal Two myelocytes of hematogenous mast cells with large nuclei were seen and tissue mast cells with small lymphocytic nuclei were fairly numerous in the older rabbit A few plasma cells and plasma mast cells were also seen There were no tissue mast cells in the thymus of the younger animal

There were many cells which appeared to be macrophages in the sections of the older irradiated animal They contained flakey granular material which stained pale blue or green with the toluidin blue of Dominici s stain and light pink or

for all showed perfect preservation Their nuclei and cytoplasm are so characteristic and specific that there was never any difficulty in identifying them Only a few of these cells had the nuclear grooves that seemed rather characteristic of the rabbit epithelial cells Many nucleoli were seen in the imprints but in the rabbit they were seen only in the sections The nuclei of the human cells are coarser and darker than those of the rabbit The cytoplasm shows about the same structure and staining reaction in both

All three types of myelocytes were seen in the human material They were most numerous in the fetus and least numerous in the 2 year old child that died of meningitis Most of the eosinophil myelocytes were of the bone marrow type and many had both basophilic and acidophilic granules in the same cell This difference in staining reaction was also seen in some of the neutrophil myelocytes These variations in staining were not seen in the rabbit thymus but were noted in the marrow A few human eosinophils had small dark lymphocytic nuclei There were no basophilic granules in these cells Most of the eosinophils of the rabbit were of this type

Some macrophages with lymphocytic nuclei were seen in the thymus of the premature infant and in a stillborn that died 1½ hours before birth from toxemia Some material that seems to have been phagocytized was included in epithelial cells of the latter case

Although the human material was obtained at autopsy done several hours after death much of it was sufficiently well preserved to show great similarity between the human and rabbit thymus There are some differences in details as in the minute structure of the epithelial cells the presence of basophilic granules in many of the human eosinophil and neutrophil thymic myelocytes and the absence from the human thymus of lymphocytic neutrophil myelocytes similar to the pseudoeosinophils of figures 14 and 15 from the rabbit

Whether the human thymus also has immature lymphocytes of the blast type could not be answered from this material There is no explanation for the absence of mesenchymatous elements like the one illustrated in figure 7 Study of more favorable material will be necessary to determine whether the human reticulum contains less mesenchyme than that of the rabbit or whether there is some other reason for its absence from the imprints

Discussion and Conclusions

It has been shown that in the normal rabbit the thymus reticulum consists largely of epithelial elements A small amount of mesenchymatous tissue is blended with this epithelial reticulum but the cells of the two types of reticulum which differ in embryological origin retain characteristic morphological differences in the adult rabbit The two types of reticular cells can be demonstrated in both sections and imprints This is contrary to the opinion of others who from the presence of fibers from embryological studies or tissue cultures of the thymus (Mietens [11] Jolly [21] Hartmann [7] Tschassonikow [36]) concluded that the thymus stroma must include some mesenchymatous elements although they could not see them in

In the material studied by the writer nothing was seen which would indicate origin of hemocytoblasts or other lymphoid cells from the epithelial reticulum However the material does show that the epithelial cells may become free rounded cells some of which assume the form of unicellular Hassall s corpuscles while others transform to macrophages or at least to cells which resemble them very closely

Human Thymus

Some human material became available during the course of this investigation It was provided by Dr Robert W Collett who performed the autopsies on infants and children who died in Minneapolis hospitals The imprints made by Dr Collett were studied by the writer Sections were not available at the time this study was made All of the material shows some postmortem change and was not good enough for detailed study of lymphocyte nuclei However it could be seen that the lymphocytes were practically identical to those of the rabbit except that the cytoplasm of the larger cells was not as basophilic It was noted that the largest lymphocytes did not equal the largest ones of human lymph nodes and that the thymocytes usually had less cytoplasm than the lymphocytes of lymph nodes

There were some lymphocytes with leptochromatic nuclei but because of the postmortem changes one could not be certain that they were true blast forms Cells from the mesenchymatous reticulum similar to figure 7 from rabbit could not be identified Cells from the epithelial reticulum however were quite numerous in some cases especially in a 2 year old male who died of influenza meningitis in a 10 weeks premature that lived 21 days and in a 5½ months aborted fetus which lived for 3 hours

Several groups of 4 to 8 attached epithelial cells were seen they were most numerous in the premature infant Most of the epithelial cells very numerous in the 2 year old child occurred as single round irregular or elongated cells The round cells generally had smooth surfaces while the margins of the elongated or irregular cells were often serrated or spiked The nuclei were elliptical in the groups of attached cells and round in most of the free cells

The nuclear membrane of all the epithelial cells was very thin Many of these cells had 1 or 2 pale blue spherical nucleoli In the largest epithelial cells the nuclei were very pale and had little chromatin which was widely dispersed in the form of fine strands and granules The nuclei of all of the smaller cells had about the same structure they were darker due to condensation of chromatin in dense short rods and irregular small masses with a considerable amount of pale pink parachromatin between them The cytoplasm was opaque and nearly homogeneous It was colored dark gray which often had a pale pink cast with May Giemsa staining In some cells it contained some blue flakes which appeared to be similar to the material included in the macrophage like cells of the older irradiated rabbit This substance was not abundant in any of the human cells nuclei were not eccentric and the cells did not resemble macrophages as they did in the rabbit

The epithelial cells resist portmortem changes much better than the lymphocytes

for all showed perfect preservation Their nuclei and cytoplasm are so characteristic and specific that there was never any difficulty in identifying them Only a few of these cells had the nuclear grooves that seemed rather characteristic of the rabbit epithelial cells Many nucleoli were seen in the imprints but in the rabbit they were seen only in the sections The nuclei of the human cells are coarser and darker than those of the rabbit The cytoplasm shows about the same structure and staining reaction in both

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histological preparations The mesenchymatous elements are identical in structure and functional potencies to the reticulum of the other lymphoid organs This tissue is not confined exclusively to perivascular regions as Popoff[23] seems to think it is although it is more abundant in these locations especially in the medulla

The present study has shown that lymphocytes and granulocytes may develop from the mesenchymatous reticulum although this type of heteroplastic development is not very active in the normal thymus and is difficult to demonstrate for lymphocytes During active regeneration following irradiation of the thymus of a young animal many examples of the heteroplastic development of lymphocytes were seen Some macrophages are also formed from free cells of this tissue

The epithelium does not form lymphocytes or granulocytes Some authors who believed that thymocytes (lymphocytes) were derived from the epithelium probably saw transitional stages between the mesenchymatous reticulum and lymphocytes This is especially true of Prenant,[19] who stated that mitosis in endodermal reticulum results in the formation of lymphoblasts with clear nuclei which divide and form lymphocytes

Free rounded or irregular shaped cells are liberated from the epithelium These were especially numerous in some of the human material where they retained all their specific epithelial characteristics In the irradiated older rabbit some of them assumed the form of unicellular Hassall s corpuscles and others appeared to be macrophages which retained some of the nuclear characters of the epithelial cells

Most students of the thymus problem agree with Rudberg[5] Ssyssojew[6] Wituschinski[7] Marine[8] and others that the epithelium can form macrophages which phagocytize degenerating lymphocytes during pathologic involution and inflammation of the organ However, Popoff[23] and Tschassownikow[36] working with transplants and cultures concluded that dye storing macrophages are derived from mesenchymatous elements only It does not seem likely that epithelial macrophages which will phagocytize lymphocytes would not also store lithium carmine It is generally agreed that the fixed epithelial cells and the free ones which have not transformed to macrophages will not store the dye

The thymocytes are true lymphocytes of various sizes and nuclear structure corresponding to those of the lymph nodes However the rabbit thymus contains a few large lymphocytes with very immature nuclei (fig 8) which resemble those of the rabbit myeloblast and lymphoblast of human lymphatic leukemia According to Sundberg and Downey[41] these are not present in the rabbit lymph nodes The thymus contains only a few transitional stages between mesenchymatous reticulum and large lymphocytes These are numerous in lymph nodes and they are usually large cells larger than any that occur in the thymus The absence of the largest lymphocytes from the thymus therefore seems due to reduced heteroplastic development of lymphocytes in the normal thymus as compared to lymph nodes The small medium and smaller large lymphocytes are identical to the corresponding forms of the nodes The smallest lymphocytes with very dark nuclei with coarse chromatin blocks and little or no cytoplasm are more numerous in thymus than in node Scanty cytoplasm is rather characteristic of the thymus lymphocytes

which seems to support the view of Dustin[19] that the nuclei are of chief importance for the function of the thymocytes

The thymic lymphocytes of different types can develop to granulocytes and they do this in the normal rabbit and human thymus They also differentiate to plasma cells and tissue mast cells There were no basophilic granules in any of the heterophil or eosinophil myelocytes of the rabbit thymus These dark granules are a conspicuous feature of these cells in the rabbit marrow and they also occur in the corresponding cells of the imprints of human thymus where most of the eosinophils are of the marrow type In the rabbit thymus all the eosinophils seem to develop from small and medium lymphocytes with dark nuclei and their granules are acidophilic when they first appear Some of the rabbit granulocytes develop from the local mesenchyme (fig 10) and this may be the origin of the marrow type of heterophil and basophil (figs 2 and 4) Heterophils like those of figures 14 and 15 could not be located in the rabbit marrow or the human thymus They are fairly numerous in the rabbit thymus

Study of regeneration of lymphocytes six days after irradiation of the thymus of a young adult rabbit showed that some of the lymphocytes are derived from the local mesenchyme under the capsule and in the medulla The intermediate stages in the process could be traced Many lymphocytes seem to enter the organ through the lymph vessels that accompany the larger blood vessels Radiation seems to interfere with the development of myelocytes in the thymus as none was found in the younger animal and only a few in the older one Irradiation was also responsible for the production of many macrophage like cells in the older animal Many of these originated from the epithelium but some had lymphocytic nuclei and others resembled the tissue type of marophage The fixed epithelium contained some included material that seemed to have been phagocytized This gives some support to those who derive true macrophages from the thymic epithelium

The development of lymphocytes from mesenchymatous tissue could not be detected in the older animal This may be because regeneration of lymphocytes was not very active in this animal Some tissue mast cells were seen in the thymus of this animal while in the younger animal the basophils were of the hematogenous type

Mesenchymatous reticular cells could not be detected in imprints of human thymus Cells of the epithelial reticulum are fairly numerous They appear in attached groups or as single round or irregular shaped cells The nuclei of all these cells are of similar structure which is characteristic of the epithelial cells Some of the nuclei have a surface groove and most have spherical nucleoli

All three types of promyelocytes and myelocytes are present in the imprints of human thymus They are similar to those of bone marrow with the exception of some of the eosinophils which develop from small lymphocytes

The mature lymphocytes are similar to those of the rabbit and as in the rabbit the largest lymphocytes of nodes do not seem to occur in the human thymus The material was not adequate for determining whether the human thymus also has lymphoblasts or reticular lymphocytes

SUMMARY

Thymocytes are genuine lymphocytes The largest lymphocytes of lymph nodes do not occur in the thymus the smallest ones with pacychromatic nuclei and scanty cytoplasm are more numerous in the thymus Some lymphoblasts similar to myelo blasts occur in the rabbit thymus

All three types of granulocytes develop from lymphocytes and to some extent from the mesenchymatous reticulum in the rabbit In the rabbit the heterophil and eosinophil granules are acidophilic when first formed while in the human thymus many have a basophilic quota as in the marrow The reticulum is largely of epi thelial origin and retains its epithelial characteristics in cytoplasm and nucleus In the rabbit some mesenchymatous tissue is blended with the reticulum and may give origin to lymphocytes and granulocytes The epithelium may form macro phages but does not differentiate to lymphocytes and other types of cells In im prints from the rabbit the epithelial cells usually can be distinguished from those of the mesenchymatous reticulum During regeneration following irradiation the latter tissue can be seen to form some lymphocytes locally other lymphocytes enter the organ through the lymph vessels

Imprints stained with May Grunwald and Giemsa are of great value for detailed cytology of the thymus and for determining cell relationships Sections of thymus fixed in Helly s fluid stained with methyl green and pyronin and dehydrated in dioxan are excellent for lymphocytes and the transitional stages resulting from their development from fixed mesenchymatous tissue during regeneration of the organ following exposure to x rays

ADDENDUM

Since this paper was written and sent to the editor an important paper on Pure cultures of rabbit thymus epithelium by R G Murray has been published (Am J Anat *81* 369 *1947*) By cutting away the main part of the explant and *leaving* only an epithelial outgrowth Murray was able to obtain a pure culture of the epithelium It was found that this epithelium grows like that from other sources At times it grew like connective tissue although mesenchymatous tissue could be excluded in this case Pinocytosis and possibly phagocytosis are characteristics not common to other epithelia True macrophages however were not formed from the thymus epithelium Evidence was not obtained for an epithelial origin of the rather numerous macrophages seen in the early and late cultures

The thymocytes behaved like true lymphocytes and some of them developed to myelocytes with lymphocytic type of nucleus

In two instances mitoses of epithelial cells resulted in the formation of one daughter cell resembling a thymocyte while the other one retained the epithelial character of the parent cell This is the only evidence obtained from the cultures in favor of a possible origin of thymocytes from the epithelium

In another recent paper R N Baillif (Anat Rec *100* 16 1948) concludes that in the involuting rat thymus after injections of colloidal chlorazol black E and

mercuric sulfide the epithelium differentiates directly to thymocytes and to macrophages which ingest the injected material Most of the new lymphocytes produced during regeneration arise through transformation of epithelial cells

It is possible that these conclusions can be explained by failure to distinguish between the epithelial and mesenchymatous portions of the thymus stroma

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A HEMATOPOIETIC PERIFOLLICULAR ENVELOPE IN THE RAT SPLEEN

By E B Krumbhaar M D, Ph D

IT IS well known that hematopoietic cells of various kinds occur normally in the spleen of rats and other rodents as well as of other animals In addition to these lymphocytes and reticulo-endothelial cells found in the Malpighian follicles and accompanying small arterioles these cells may also be found in the pulp, together with erythrocytes and occasionally neutrophils eosinophils plasma cells megakaryocytes myelocytes and nucleated erythrocytes (Jaffe[1]) These cells are usually described as occurring diffusely or in small clumps It has apparently escaped general notice however that surrounding the Malpighian follicle is an envelope of hematopoietic cells which is fairly well circumscribed lacks sinuses is separated from the follicular cells by a shell or rind of connective tissue and responds to hematopoietic stimuli differently than does the red pulp Under certain pathologic conditions it may appear to be more voluminous than the follicle which it surrounds To be sure Jaffe speaks of a *Follikelhof* or *Aussen one* from which the follicle is sharply separated which Weidenreich[4] called a *Rand one* and Strasser a Follikdaussenzone Strasser also noted absence of sinuses and a thin rind Also in a study of the toxicity of benzene in rats and other animals Svirbely Dunn and Von Oettingen[3] noted narrowing of the perifollicular collars of closely packed pale cells Dr R D Lillie in whose laboratory this work was performed writes that he conceives of this zone as possibly the pressure reducing mesh homologous to the ellipsoids of dogs and cats Its conspicuousness is quite variable To me [R D L] it has seemed to vary inversely with the blood content of the pulp and sinuses It is often the principal site of hemosiderin accumulation As will appear below we too have found the area to vary greatly in size and content under different conditions and in general to have its size modified by the amount of blood in the red pulp However under our conditions at least it seems to be rather an auxiliary hematopoietic tissue, depleted when exposed to hematopoietic poisons and exuberant during periods of active blood cell regeneration

Present Study

The preparations studied were mostly routine paraffin cut sections (6–8 micra in thickness) stained with hematoxylin and eosin Other stains such as eosin methylene blue and azur 2-eosin were also used Details of the records of the experiments tried leukocyte and differential counts and the weight and gross appearance of the spleen were of considerable help in evaluating the findings During the examination of the hematopoietic system of several hundred rodents chiefly adult rats during the course of a study of the pathologic effects of mustard gas and other hematopoietic poisons it soon became apparent that the spleen contained two rather distinct hematopoietic areas in addition to the lymphocyte forming Malpighian

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follicles These were (1) small foci scattered through the red pulp (2) the perifollicular areas already referred to The small foci were apparently composed of either myelocytic erythrocytic monocytic or lymphocytic cells or sometimes of mixtures In the case of the lymphocytes they may have been about small vessels which, as Jaffe notes can be brought out with the perfused specimens the cells of some of these foci have the same appearance as those in the envelopes about to be described As might be expected these foci tended to disappear in the spleens depleted by poison, and to be large and widespread in recovery periods during active

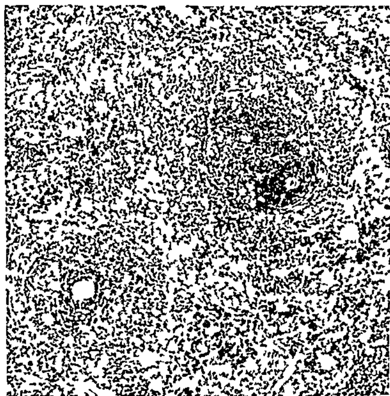


Fig 1 Normal rat s spleen Two Malpighian follicles (MF) with their central arteries appear surrounded by the paler staining perifollicular envelope (PFE) The more open red pulp occupies the rest of the field (H E 150 ×)

regeneration However as so often is the case with different units of the hemolymphopoietic system in the present state of our knowledge they at times showed unexpected and unexplained responses

In sections of normal rats spleens the perifollicular areas are easily distinguished in the stained specimen as collars of nucleated cells paler and larger than the adult lymphocytes of the Malpighian follicle The collar a term appropriate for the two dimensional section but not for what is obviously a three dimensional structure varies in width from about 30 to as much as 200 micra It is separated from the follicle by a delicate but definite line of collagenous connective tissue which is

presumably a development from the follicular framework. This narrow rind contains a young type of fibroblast and sparse collagen fibers. The rind usually cannot be traced around the complete circumference. In one instance (rat 1630) a capillary was enclosed in this band for about one quarter of the perimeter. The cells of the perifollicular areas in normal spleens are mostly mononucleated, about the size of young lymphoblasts, the nuclei round and moderately full of chromatin, often with a deep staining central dot, and with scanty pale staining cytoplasm. They show

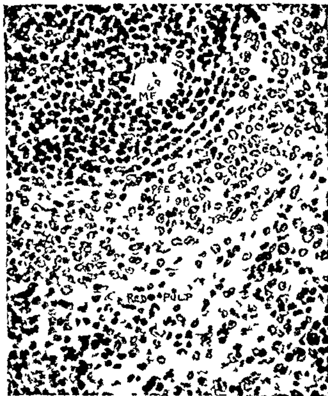


FIG 2. Same section showing part of the larger follicle and its collar. Note the curved rows of adult lymphocytes near the margin, separated by delicate connective tissue (which does not show in the photograph). The more homogeneous nature of the collar than of the red pulp is obvious. (H E 320 X)

no gradation to the fibroblasts of the thin rind, nor do they resemble them in appearance.

They do not appear to be monocytes, as they have scanty cytoplasm that is not granular and never contains ingested material. The nuclei are never indented and show no characteristic nucleoli.

These cells were further investigated by making imprints of the freshly bisected surface of some 30 normal rats' spleens and comparing the stained imprint (May Grunwald Giemsa) with stained sections from the other side of the bisection. Successive imprints, a dozen or more per spleen, reproduced the same appearance

with surprising similarity, given a marked if sup-rficial resemblance to serial sections Though the blood cells did not stain as well by this method as they do in good blood smears, the cells in question were easily identified They were thought to be young lymphocytes by several colleagues as well as by myself Compared to the small dark staining nuclei of adult lymphocytes and normoblasts, in the imprints their nuclei were 50 per cent larger paler, with coarse chromatin clumps and a tendency to condense around the nuclear membrane The cytoplasm was mostly very scanty where more abundant it stained a very pale blue without granules or

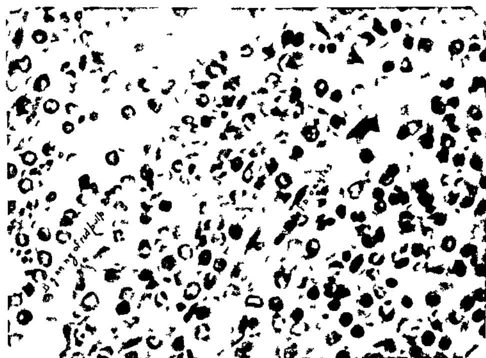


Fig 3 Greatly depleted spleen of rat 6 2 7 dying seventeen days after nitrogen mustard gas poisoning Both the follicle and collar have lost many cells with a number of polys immature red cells and debris present Note the fibrocytes separating follicle and collar (H E 135 ×)

vacuoles Oxidase granules (benzidine stain) could not be demonstrated in their cytoplasm such as appeared in the neutrophils

The possibility that these cells are immature monocytes or myeloid cells cannot be ruled out until studies can be made under living conditions as in tissue cultures and such items as their mode of growth and locomotion observed Occasional larger round heavily stained nuclei (immature cells) are found and more sparsely and not constantly normoblasts and adult erythrocytes polymorphonuclear neutrophils and eosinophils also large pale vesiculated nuclei that are taken to be adult reticulo endothelial cells No megakaryocytes have ever been found in the collar and no sinuses The outer margin of the zone merges almost imperceptibly into the red pulp the transition being chiefly determined by the appearance of

sinuses and more stroma and by the much greater number of erythrocytes Sections that cut through the edge of a follicle may show a broader perifollicular than follicular area or even none of the adult lymphocytes of the true follicle at all

In the spleens depleted by various poisons of the lymphocytic and granulocytic cell series the perifollicular collar may be represented by an almost empty circular band (except for an extremely scanty stroma) bounded on its two margins by a still recognizable follicular rind and the denser stroma of the red pulp If the damaged spleen is congested, the empty collar stands out in bold contrast

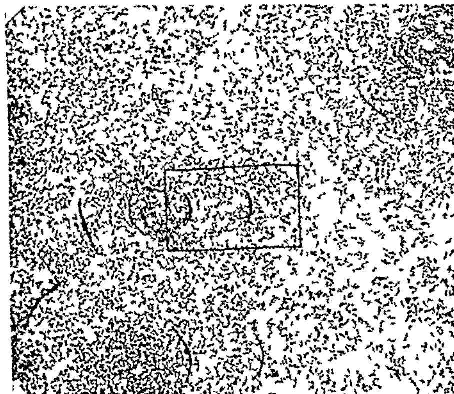


FIG 4 Spleen of rat 6-t 3 kill d w hile recovering from a non lethal dose gi en three w ks earlier Note the extremely wide collar of a follicle that is elatively small The rectangl indi ates the field of the next figure with an approximation of the width of some collars indicated (H E 150 X)

In spleens of rats killed three weeks to three months after the experiments the collars usually but not always appear larger and paler than normal with larger cells and occasional mitoses present More hematopoietic cells of several kinds except the megakaryocyte are found interspersed and sometimes in concentrated foci that suggest local production As the collars contain only few erythrocytes the more the red pulp is congested the more conspicuous the relatively pale reddish purple collars appear between the dark blue of the follicle and the red of the erythrocytes In rare instances however hematocytic regeneration in the pulp foci may be so

extensive that more erythrocytes are found diffused through the collars together with their usual cells than in the pulp which is hardly recognizable as splenic tissue

Turning to other rodents—in the mouse spleen the collar is less developed nucleated cells are much more frequent throughout the pulp and the transition from follicle more gradual The collar therefore is almost indistinguishable though occasional suggestions of a connective tissue rind may be seen beyond which the *lymphocytes are more condensed than farther out in the pulp Here too the numer*ous megakaryocytes in the pulp aid in the differentiation

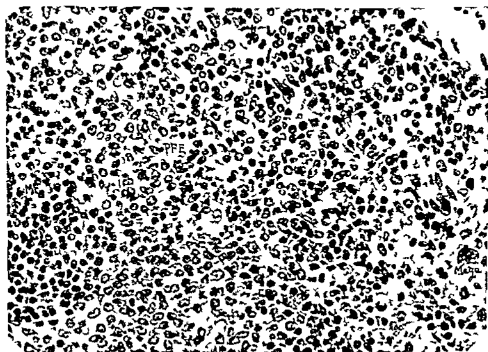


Fig 5 Same section (800 X) The beginning of the red pulp area is hard to distinguish though the megakaryocyte (mega) sinuses (s) and RBC (to the right) are surely in the red pulp (H E)

In the rabbit the picture is more like that of the rat except that pale centers (reticulo endothelial cells?) may often be found in the topographic center of the follicle they are rare in the rat The rind of connective tissue is usually found the limits of the follicle being indicated both by this pink staining strip and by a tendency for the sectioned tissue to separate at this place The cell population of the collar varies as in the rat with a fair number of nonlymphocytic cells that are not to be found inside the rind

In the guinea pig which may have pale centers in the follicles both rind and collar are scanty and ill defined indeed even their existence in places seems doubtful though Jaffe says the collar is easy to find if perhaps narrow When found it has the same difference in cell population from the follicle as does that of the rat

In a few normal hamsters the perifollicular tissue is also found to be less constant

and rich than in rats Few if any myeloid cells are seen ripe or unripe and very few normoblasts and erythrocytes The rind varies much in size not being found at all in small follicles

Summary and Conclusions

About the Malpighian follicles of several species of rodents and especially prominent in rats is a perifollicular envelope composed of hematopoietic cells that takes active part in hemolytopoietic changes

The identity of the mononuclear cell comprising the greater part of this tissue has not been positively determined it is probably a young lymphocyte It is possibly the homologue of the pale centers of the Malpighian follicles in man and other mammals though it has been found in rabbit spleens which also have pale centers It should be possible to determine this identity by the use of a greater variety of stains and of test poisons and if possible of dynamic methods such as tissue culture and moving pictures

The envelope is separated from the Malpighian follicle by a thin rind of collagenous connective tissue but on its outer margin it merges gradually with the red pulp It often contains a scattering of erythrocytes normoblasts polymorphonuclear neutrophils and rarely eosinophils and pigment bearing macrophages Some of these cells were so greatly increased under the pathologic conditions first studied that colonization was suggested they were later thought to have probably wandered in The collar never contains megakaryocytes or sinuses or blood vessels of any noteworthy size

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THE PLACE OF THE SPLEEN IN THE ENDOCRINE SYSTEM

By Ardrey W Downs M D

FROM being considered as of no use to the organism the spleen has come to occupy a rather insecure position as a gland of internal secretion The purpose of this article is to try to show how this change has come about A wide variety of functions has been assigned to the spleen from time to time most of them involving the life cycle of the blood corpuscles The spleen has been stated to manufacture red blood corpuscles and on the other hand it has been claimed that red corpuscles are destroyed in this organ In the same way the spleen has been regarded by some as a place where white blood corpuscles are produced and by others as a place where they are destroyed Evidence of an endocrine function of the spleen is still meagre and to some extent conflicting

It is necessary to touch briefly on the background The spleen was known to the ancients the Greeks considering it as inessential to life Aristotle refers to it and Erasistratus states that it is useless It was regarded by the Greeks and Romans as detrimental to a runner and this idea persisted at least to the time of Shakespeare It is frequently stated that the ancients removed the spleens of runners to increase their speed

Splenectomy is said to have been performed in Europe in the sixteenth century Moynihan[1] gives an account of the operation performed by Zaccarelli as written by an Italian physician Leonardo Fioravanti for the removal of the spleen from a woman 24 years old Apparently she made a prompt recovery and suffered no ill effects Moynihan points out that the suggestion has been made that the mass re moved was an ovarian cyst At least two other removals of the spleen are stated to have taken place during this century During the seventeenth century two sple nectomies are recorded both of which seem to be reasonably well authenticated In both cases the reason for the operation was knife wound in the left side with prolapse of the spleen

The first experiments known to have been performed on animals in a study of the spleen as described by Moynihan were carried out by Malpighi in 1669 Clarke in 1676 and Zambeccari in 1680 Malpighi ligatured the splenic artery and vein in a dog Subsequently the spleen underwent complete atrophy and the liver enlarged Both Clarke and Zambeccari performed splenectomies on dogs No significant change was observed

In 1841 Bardeleben[2] published the results of the first carefully planned experi ments directed toward elucidation of the function of the spleen The results of com plete removal of the organ were briefly these a transitory decrease in the number of red and an increase in the number of white corpuscles in the circulating blood an increase of activity of the bone marrow and lymphatic glands no apparent ill effect on life These experiments are particularly noteworthy because of the care with

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which they were planned the thoroughness with which they were carried out and the conclusions drawn

It was at this time that general consideration began to be given to removal of the spleen as a therapeutic measure The results of Bardeleben s experiments gave support to the belief that the spleen was not necessary and splenectomy came to be employed more and more Collier[4] recounts 29 cases of splenectomy reported to 1882 Of these 13 were for wandering spleen enlargement (described as simple) and hydatid cysts Eight of these were regarded as successful In the other 16 cases leukocythemia was present None recovered These results made it clear that splenectomy was neither a sound nor safe procedure for leukocythemia

A relationship of the spleen to hematopoiesis was recognized as a result of Bardeleben s experiments which were confirmed by other investigators The unsuccessful outcome of extirpation of the spleen in leukocythemia led to a turning of attention to the anemias At the beginning of the twentieth century any syndrome of splenic enlargement and anemia was classified as a splenic anemia If the spleen were responsible for destruction of red blood corpuscles then it seemed to follow that a condition such as pernicious anemia should be benefited by removal of the organ Consequently during the second decade of the century splenectomy was resorted to in a number of these cases The results were inconclusive

During this time Whipple Hooper and Robscheit had been doing fundamental work on the anemias Their report[5] on the influence of meat liver and various extractives on blood regeneration following simple anemia in dogs produced by bleeding is the foundation on which was developed our present knowledge of blood formation From this study came the use of liver in pernicious anemia Minot and Murphy[6] found that feeding one half pound of liver daily brought about improvement in patients with pernicious anemia It later became clear that the fraction of liver which was curative in pernicious anemia was not the one which was effective in the anemia of bled animals These discoveries led to the abandonment of the surgical procedure of splenectomy as a mode of treatment of anemia

With the rising interest in internal secretions the spleen was not neglected though it did not receive as much or so wide spread attention as some other endocrine glands During this period among the most notable workers was the group headed by Pearce with Musser and Krumbhaar and a large number of associates Among others on this continent who were interested in trying to establish something definite as to the way in which the spleen functions were Leake Holloway and Blackford Eddy and Downs In Europe probably Danilewsky Stradomsky and Mouzon were particularly interested in the physiology of the spleen

Investigation of the part that the spleen plays in the formation and in the destruction of blood cells has followed largely four lines Microscopic examination of the spleen the counting of the cells in the blood going to and coming from the organ the results of splenectomy and the effects of administration of splenic subtance or extract

During a period of about fourteen years centering on the second decade of this century Pearce and his associates carried out many experiments intended to throw light on the function of the spleen Dogs were the animals used In 1913 Musser and

Krumbhaar[7] stated that after splenectomy anemia usually develops quickly and reaches its height in from three to six weeks then the blood picture approaches the normal after about three to four months with complete return to normal in five to ten months Accompanying this is marked leukocytosis which reaches its height in twenty four hours but persists to a slight degree for several months In 1912 Karsner and Pearce[8] had reported an increased resistance of red blood corpuscles after splenectomy This was confirmed the following year by Pearce and Peet[9] who stated further, that the increased resistance cannot be explained on the basis of an increase in reticulated cells in the circulating blood Practically all observers agree that after splenectomy the red blood corpuscles are less fragile than normally Pearce Krumbhaar and Frazier[10] had concluded that the transient anemia following splenectomy is due to the loss of some substance that stimulates the bone marrow with a lack of blood formation rather than to increased blood destruction This conclusion was supported by the observation of themselves and others that it was relieved by the administration of splenic extract

In 1920 Downs and Eddy[11] published results of the subcutaneous injection of single doses of splenic extract in rabbits on the number of red corpuscles in the circulating blood The immediate effect was a temporary decrease in the number It was thought that the decrease might be due to a direct hemolytic action of a splenic agent There was frequently a very transient increase in the number of white corpuscles In 1921 Eddy[1] enunciated the hypothesis that the spleen produces an internal secretion This was based on the changes in the erythrocytes after splenectomy the modification of the blood picture in hyperplasia of the spleen and the specific effects on the red blood corpuscles of injection of splenic extract Nothing was known of the chemical nature of the supposed hormone and it was difficult to formulate a consistent theory of its possible mode of action He suggested that the chief function of the spleen is the removal from the circulation of disintegrated erythrocytes that the splenic cells elaborate this material and thereby produce an internal secretion that this internal secretion possibly after modification by the liver stimulates the erythrogenic function of the bone marrow and is used up in the manufacture of new corpuscles

Danilewsky[13] in 1895 was able to cause a marked increase in the number of red corpuscles in the circulating blood and also in the hemoglobin content of the blood by a single intraperitoneal injection of an extract of the spleen Apparently he was the first to suggest that the spleen acts on the bone marrow In 1916 Stradomsky[14] had concluded that the immediate effect of the splenic agent was destruction of erythrocytes The increase in production of red blood corpuscles by the bone marrow however went beyond the usual response to a reduction in the number of corpuscles in the circulating blood and he felt that this was explained by assuming the removal of a normal regulating action by a splenic hormone on the bone marrow

In 1922 and 1923 Downs and Eddy[15, 16] reported further experiments in which splenic extract was administered subcutaneously to rabbits daily for periods of from four weeks to fifteen weeks These showed the appearance of reticulated cells in the circulating blood in a proportion much greater than normal the presence o

nucleated red corpuscles in the circulation and an increase in the resistance of the circulating red blood corpuscles These results agreed with those obtained previously and appeared to confirm the theory of splenic action that had been proposed

At this time Leake and Leake[17] demonstrated that both extract of spleen and extract of red bone marrow are hematopoietic agents and that a combination of the two is more powerful than either one alone In their opinion they act first by increasing the rate of production and second by causing an extension of functioning red marrow Leake and Evans[18] followed the treatment of various types of anemia in humans by the use of desiccated spleen and red bone marrow combined in equal quantities Improvement was obtained in grave secondary anemias both active and chronic in dietary anemias of infants and in menorrhagic anemias

The development of the concept of the spleen as a gland of internal secretion has taken place gradually during the past quarter century During later years it has been based almost entirely on pathologic and clinical observations In 1916 Kaznelson[19] showed that removal of the spleen was followed by a rise in the platelet count and clinical improvement in some cases of thrombocytopenic purpura He concluded that the spleen had been exerting an excessive cytolytic action on platelets Whether Kaznelson s conclusion was sound or not his recommendation that splenectomy be performed in cases of thrombocytopenic purpura was followed by the report of excellent results in 16 cases [20] Troland and Lee [1] in 1938 described a substance obtained from spleens that had been removed from patients suffering from thrombocytopenic purpura which caused a reduction in the platelet count when injected into animals Wiseman and Doan[2] in 1942 described a condition that they ascribed to hyperactivity of the spleen The spleen was enlarged and the white blood corpuscle count low They named it primary splenic neutropenia Splenectomy was followed by an increase in the leukocyte count and improvement in the condition of the patient They believe excessive activity of a splenic hormone to be the cause without being able to determine what has led to this disturbance of splenic processes In 1946 Doan and Wright [3] described primary splenic panhematopenia in which there is a reduction of all formed elements of the blood red corpuscles white corpuscles and platelets In these cases also splenectomy caused marked improvement

This study of the spleen is not unmindful of the useful purpose served by the spleen as a reservoir of quickly available red blood corpuscles with their hemoglobin Nor does it overlook the phagocytic activity of the gland It is however concerned with a different problem Moreover it has been pointed out that the phagocytic action and the production of one or more agents affecting the red blood corpuscles in active circulation and the bone marrow may be closely related

Conclusion

An attempt has been made to trace the scientific study of the function of the spleen from the time of ancient Greece to the present Modern theory and experimentation have linked the organ with the formed elements of the blood Gradually a theory of endocrine activity has been developed which relates the spleen to the corpuscle content of the circulating blood A normal blood picture is due in part at

least to a normally functioning spleen There is then the possibility of hyper or hypoactivity and it seems reasonable to regard certain clinical entities as due either partly or wholly to disordered splenic activity Those conditions in which it seems to be fairly well established that removal of the spleen should be considered are thrombocytopenic purpura splenic neutropenia and primary splenic panhematopenia Cases so far reported suggest strongly that the prognosis is much improved by splenectomy What the later effect of absence of the spleen may be remains unknown A clinical manifestation of hypoactivity of the spleen does not appear to have been recognized as yet

Summary

While the evidence for an endocrine function of the spleen is meagre and the exact nature of this action is not clear it does seem to be fairly well established that the spleen must be considered as an important part of the mechanism whereby a normal corpuscle and possibly platelet content of the circulating blood is maintained

This regulation seems to be due to the production by the spleen of one or more hormones

These hormones appear to affect the processes of the bone marrow and also may act upon the corpuscles in the circulating blood

The conception of hyperactivity of the spleen in the human being seems to be firmly established but little is known of the clinical appearance of its hypoactivity

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THE EFFECT OF ENDOCRINOPATHIES ON THE BLOOD

By William H Daughaday M D Robert H Williams M D
and Geneva A Daland B S

With the rapid increase of knowledge of the many functions and interrelations of the endocrine glands it is natural that their influences on the formed elements of the blood have received extensive study The essential problem has been to determine just how much the elements of the blood are under direct endocrine regulation and how much they are affected by general metabolic alterations produced by hormones Attempts to isolate a specific hemopoietic hormone or hormones have not been successful Extracts of the anterior pituitary gland have been said to aid in the regulation of erythrocyte production[1] and certain steroids of the adrenal cortex are known to affect lymphoid tissue In this paper we propose to review some of the clinical and experimental evidence pertaining to this subject and to present some of our observations in an attempt to evaluate the importance of hormones in the regulation of blood production

1 Gonads

A sex difference in the number of red blood cells and the concentration of hemoglobin has been well established for human adults Exact figures vary but all authors agree that the values for the male are significantly higher than for the female One authority[2] states that the red blood cell count averages 4 8 million in females and 5 4 million in males The concentration of hemoglobin shows a corresponding difference This difference cannot be ascribed to blood loss in menstruation because a wide variety of mammals and even birds appear to have a well substantiated sex difference

A mild anemia occurs after castration in the male hamster[4] rabbit[5] rat[6, 7] and chicken[8] The anemia is usually slightly hypochromic and microcytic The administration of androgens in general has proved effective in restoring the red blood cell counts to normal or above normal McCullagh and Jones[9] have found a slight to moderate reduction in erythrocytes and hemoglobin in eunuchoid men They showed that treatment with testosterone caused a rise in the red blood cell counts and hemoglobin with cessation of therapy there was a reversal of these changes An increase in the basal metabolic rate seemed to parallel the improvement in the blood picture From these data they concluded that the sex difference in basal metabolic rate and in erythrocytes might be a related phenomenon

Ovariectomy of rats causes a rise in the red blood cell count and hemoglobin to nearly the levels maintained by castrated males[6] The administration of estradiol to these animals yields values comparable to those of normal female animals The chicken seems to respond somewhat differently in that the red blood cell count of

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ovariectomized hens is not significantly different from that in the hen [8] A depression or erythropoiesis follows large doses of estrogens in several species A moderate to severe anemia has been produced in dogs with both natural and synthetic estrogens [10-1] The depression is not limited to the red cell series but a severe and sometimes fatal granulocytopenia or thrombocytopenia will occur on continued treatment Monkeys seem to be much more tolerant of similar doses and show only a slight anemia [1 13] Hematologic complications of estrogen therapy in humans seems to be rare *

There is not universal agreement as to sex differences in the number of platelets A slightly lower average platelet count has been reported for women by Pohle [14] In 13 normal women it was found that a gradual decrease in platelets occurred during the fourteen days prior to menstruation which was followed by a rapid return to normal or increase *after the onset of menstruation Purpura hemorrhagica* is found more frequently in females and cases of thrombocytopenic purpura have been described in which the purpuric episodes recurred only at the time of menstruation [15] These observations suggest that platelets are influenced by certain female sex hormones or perhaps by the menstrual toxin described by Smith and Smith [16]

2 Thyroid

Considerable clinical and experimental evidence indicates that the thyroid hormone has a definite influence on hematopoiesis An anemia occurs with regularity in many laboratory animals following complete thyroidectomy The changes which occur in the rat[17] and rabbit[18 19] have received the most thorough study The characteristic picture is a moderate anemia which is normochromic and slightly macrocytic Gastric acidity in the rabbit is unchanged and megaloblasts do not occur in the bone marrow A diminished ability to regenerate red cells and hemoglobin following a standardized hemorrhage has been demonstrated in thyroidectomized rats [0] The defect was corrected by thyroxine cobalt and testosterone suggesting that the disturbance in blood regeneration is nonspecific

Anemia is frequently observed in patients with myxedema Emery [1] attributed the first definite description of this feature of hypothyroidism to Charcot in 1881 This was seven years after Gull s original paper describing the disease The report of the London Clinical Society on myxedema in 1888 added that allied with the fall in body temperature are changes in the blood There is not only anemia due to loss of corpuscles but the relative proportions of these constituents are also altered Following these early observations many other authors have confirmed and extended knowledge of this anemia The importance of the recognition of this cause of anemia was emphasized in 1921 by Dr Minot [3] in a clinic at which he presented two curable cases of anemia The changes in the blood were summarized and the beneficial effects of treatment were described In commenting on the etiology of the anemia he said The anemia in this case was apparently dependent upon a decreased formation of blood This decreased activity of the marrow is

One patient has been found to have repeated attacks of agranulocytic angina manif st d on the first day of the menstral cycle [81]

entirely consistent with the diminished activity of the other functions of the body

Stern and Altschule[1] have described the blood changes in human beings under conditions which approach in simplicity the animal experiments referred to above Their patients were subjected to total thyroidectomy for the relief of angina pectoris or congestive heart failure An anemia of some degree was common and the onset of anemia seemed to coincide with the drop in the basal metabolic rate There was a slight increase in mean cell volume and in color index Some decrease in white blood cell counts occurred but the differential counts remained unchanged

Anemia in spontaneous myxedema has been found by Bomford[3] to be of three types A slightly macrocytic variety of moderate severity commonly occurs Similar to the anemia following thyroidectomy it is characterized by a slight macrocytosis and increase in color index It differs from pernicious anemia in that there is little poikilocytosis or anisocytosis of the erythrocytes and the bone marrow is hypoactive Gastric function may or may not be normal No reticulocyte response follows treatment with liver or iron but the anemia slowly disappears on prolonged treatment with desiccated thyroid

Some cases of myxedema may be associated with a hypochromic anemia of varying degree Splenomegaly a smooth tongue and changes in the nails are sometimes observed The blood smear resembles iron lack anemia with the exception that the cells tend to be larger Achlorhydria is common but not invariable A reticulocyte response to iron occurs but complete recovery depends on both iron and thyroid

Quite rarely Addisonian macrocytic anemia may be a complication of hypothyroidism In such patients the signs and symptoms of pernicious anemia and combined system disease may be superimposed on the features of myxedema The blood resembles pernicious anemia except that the color index may be even higher and the cells larger Maximum improvement depends upon combined liver and thyroid treatment

Bomford has concluded that the simple macrocytic type is the result of a decrease in size of the erythron as a physiologic compensation for the diminished need of the tissues for oxygen The bone marrow undergoes hypoplasia with shrinkage of its total volume A marked reticulocytosis following treatment is not to be expected as compared to the anemias due to a maturation arrest The slow return of the peripheral blood to normal values was explained by the gradual resumption of activity and cellularity in the bone marrow The other two types of anemia are due to deficiencies of iron and liver extract factor apparently dependent on defective gastrointestinal function

The simple slightly macrocytic anemia of myxedema occasionally is mistaken for pernicious anemia However little variation in size and shape of the red cells occurs in myxedema and the multilobed polymorphonuclear leukocytes and bone marrow changes associated with pernicious anemia are absent The finding of normal gastric juice occurs in about one half of the cases of myxedema The basal metabolic rate is of considerable aid in diagnosis because it is usually elevated in pernicious anemia A yellow color of the skin may be common to both diseases but

this pigment is bilirubin in pernicious anemia and excess carotene in myxedema The most important differential point however is the presence or absence of an adequate reticulocyte response to liver extract therapy

The frequency of anemia in spontaneous myxedema has been reported by Lerman and Means [6] Sixty per cent of 52 patients with myxedema had red blood cell counts of less than four million and 52 per cent had hemoglobin concentrations which were le s than 70 per cent Achlorhydria occurred in 53 per cent and was more frequently associated with anemia than was normal gastric acidity These authors ascribed considerable etiologic importance to these changes in gastric secretion The coexistence of myxedema and pernicious anemia seems to be more common than could be accounted for on the basis of probability Means Lerman and Castle [7] have described 5 such cases responding to liver extract

Testosterone has been used in the treatment of anemia in myxedema by Glass [8] He reported the case of a 71 year old man with the classic features of myxedema The red blood cell count was 2 8 with a color index of 1 1 Liver and iron had been given in adequate doses without increasing the number of erythrocytes Four months of therapy with desiccated thyroid failed to correct the anemia On the addition of testosterone and methyl testosterone to the therapeutic regime the erythropoietic response was prompt and blood counts returned to normal in the course of a few months An adequate hematologic response to desiccated thyroid might have eventually occurred in this patient but the stimulating effect of testos terone on erythropoiesis cited above and the observed low excretion of 17 keto steroids in myxedema provide a rational basis for such therapy We have employed the combination of testosterone and desiccated thyroid in myxedema with apparent acceleration of blood regeneration as illustrated by the following case

Normocytic slightly hypochromic anemia associated with myxedema with restoration of normal blood values after treatment with desiccated thyroid testosterone propionate and ferrous sulfate

Case 1 M V a housewife aged 47 of Irish parentage entered the Boston City Hospital in May 1945 complaining of weakness which appeared at the birth of her last child five years previously The baby had been born at full term and the delivery was uncomplicated Lactation was normal and menstrual periods returned at normal intervals following the cessation of lactation She did note howe er in creased fatigue and weakness Somnolence and lethargy became troublesome For one year she had noticed thinning of her hair and dryness of her skin Bowel movements had continued to be regular There had been no shortness of breath numbness or tingling of the extremities and no soreness of the tongue

On physical examination the patient was a pale somewhat poorly developed middle aged female in no distres The skin was cool and dry and the fingernail were brittle and spoon shaped The hair on the scalp was thin and dry and the axillary and pubic hair was sparse The tongue was large but possessed normal papillae The blood pressure was 101 mm of Hg systolic and 75 diastolic and the pul e was 94 The heart and lungs were not abnormal The li er and spleen could not be palpated No abnormal neuro logic signs were noted

Laboratory examinations showed the following Repeated urinalysis demonstrated only a small amount of albumin on occasions without other abnormality The serum cholesterol was found to be 204 mg per 100 cc The prothrombin concentration was 90 per cent of normal Carotenoids were demon strated in the serum by a presumptive test The basal metabolic rate was −36 per cent An insulin toler ance test using 2 5 units of insulin intravenously did not reveal sensitivity or hypoglycemic unrespon siveness A water test[29] and a sodium deprivation test[30] were normal suggesting normal adrenal function The sella turcica was normal by x ray

Examination of the blood showed a red cell count of 2 98 million and the hemoglobin concentration

was 48 per cent The white blood cell count was 7 200 and the differential count showed the following polymorphonuclear neutrophils 64 per cent band forms 6 per cent eosinophils 1 5 per cent basophils 0 5 per cent small lymphocytes 15 per cent large lymphocytes 3 5 per cent monocytes 7 5 per cent and myelocytes 2 0 per cent The platelets appeared normal

The patient was given a diet of about 2 500 calories supplemented with vitamins in the form of Vegex 90 cc per day 2 5 mg of thiamin 50 mg of ascorbic acid and 50 mg of nicotinic acid a day Desiccated thyroid was begun soon after entry in a dose of 32 mg and was later increased to 96 mg Ferrous sulfate was administered in a daily dose of 0 9 Gm for a week Two cc of purified liver extract (*Lilly*) *containing* 30 *USP units was given in a single dose by intramuscular injection Five cc of crude* liver extract (Wilson) was administered intramuscularly daily for five days No significant increase in

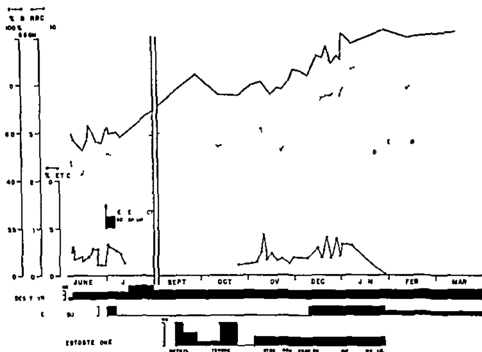


Fig 1 Effect of Therapy on the Red Blood Cell Count and Hemoglobin Concentration in Case 1

reticulocytes was observed following any of the above therapies The time relations of the various medications and the hematologic response are shown in figure 1

At the time of the patient s discharge from the hospital the red blood cell count was 2 9 million and the hemoglobin concentration as 56 per cent She had noted improvement in strength and general well being The basal metabolic rate had risen to −18 per cent She was followed at intervals in the out patient clinic From September 13 to October 27 1945 she received a total of about 1400 mg of methyl testosterone in d ily doses of 10–50 mg without improvement in her blood In November 1945 she was readmitted for more intensive treatment At this time it was noted that axillary hair was not present and that the spoon shaped deformity of the nails was absent The possibility of gastrointestinal bleeding as a cause fo her refractory anemia was in estigated Repeated stool examinations were negative for occult blood The basal metabolic rate was plus 10 per cent In addition to desiccated thyroid testosterone

One hundred per cent hemoglobin is equivalent to 15 6 Gm per 100 cc for all determinations from this laboratory

this pigment is bilirubin in pernicious anemia and excess carotene in myxedema The most important differential point, however, is the presence or absence of an adequate reticulocyte response to liver extract therapy

The frequency of anemia in spontaneous myxedema has been reported by Lerman and Means [26] Sixty per cent of 52 patients with myxedema had red blood cell counts of less than four million and 52 per cent had hemoglobin concentrations which were less than 70 per cent Achlorhydria occurred in 53 per cent and was more frequently associated with anemia than was normal gastric acidity These authors ascribed considerable etiologic importance to these changes in gastric secretion The coexistence of myxedema and pernicious anemia seems to be more common than could be accounted for on the basis of probability Means Lerman and Castle [7] have described 5 such cases responding to liver extract

Testosterone has been used in the treatment of anemia in myxedema by Glass [8] He reported the case of a 71 year old man with the classic features of myxedema The red blood cell count was 2 8 with a color index of 1 1 Liver and iron had been given in adequate doses without increasing the number of erythrocytes Four months of therapy with desiccated thyroid failed to correct the anemia On the addition of testosterone and methyl testosterone to the therapeutic regime the erythropoietic response was prompt and blood counts returned to normal in the course of a few months An adequate hematologic response to desiccated thyroid might have eventually occurred in this patient but the stimulating effect of testosterone on erythropoiesis cited above and the observed low excretion of 17 keto steroids in myxedema provide a rational basis for such therapy We have employed the combination of testosterone and desiccated thyroid in myxedema with apparent acceleration of blood regeneration as illustrated by the following case

Normocytic slightly hypochromic anemia associated with myxedema with restoration of normal blood values after treatment with desiccated thyroid testosterone propionate and ferrous sulfate

Case 1 M V a housewife aged 47 of Irish parentage entered the Boston City Hospital in May 1945 complaining of weakness which appeared at the birth of her last child five years previously The baby had been born at full term and the delivery was uncomplicated Lactation was normal and menstrual periods returned at normal intervals following the cessation of lactation She did note however increased fatigue and weakness Somnolence and lethargy became troublesome For one year she had noticed thinning of her hair and dryness of her skin Bowel movements had continued to be regular There had been no shortness of breath numbness or tingling of the extremities and no soreness of the tongue

On physical examination the patient was a pale somewhat poorly developed middle aged female in no distress The skin was cool and dry and the fingernails were brittle and spoon shaped The hair on the scalp was thin and dry and the axillary and pubic hair was sparse The tongue was large but possessed normal papillae The blood pressure was 101 mm of Hg systolic and 75 diastolic and the pulse was 94 The heart and lungs were not abnormal The liver and spleen could not be palpated No abnormal neurologic signs were noted

Laboratory examinations showed the following Repeated urinalysis demonstrated only a small amount of albumin on occasions without other abnormality The serum cholesterol was found to be 204 mg per 100 cc The prothrombin concentration was 90 per cent of normal Carotenoids were demonstrated in the serum by a presumptive test The basal metabolic rate was −36 per cent An insulin tolerance test using 2 5 units of insulin intravenously did not reveal sensitivity or hypoglycemic unresponsiveness A water test[29] and a sodium deprivation test[30] were normal suggesting normal adrenal function The sella turcica was normal by x ray

Examination of the blood showed a red cell count of 2 98 million and the hemoglobin concentration

In the group with thyrotoxicosis there were 33 patients A moderate reduction in red cells and hemoglobin occurred in 10 The white blood cell counts were low or normal and a relative granulocytopenia and lymphocytosis were found on smear The lymphocytic infiltration of the thyroid gland which was examined after surgical extirpation was correlated with the degree of lymphocytosis Only minor changes suggesting greater immaturity of the predominant cells were found in aspirations of sternal marrow

Treatment with iodine was followed by a return toward a more normal differential white blood cell count but the three groups responded to an operation with about the same changes in the blood The author concluded that the changes occurring in thyrotoxicosis were slight and inconstant and could not be of aid in diagnosis or in estimating prognosis Other recent reports agree with this thesis [33,34]

Probably associated with the lymphocytosis which may occur in thyrotoxicosis is the hyperplasia of lymphoid organs which occasionally is present A generalized enlargement of the lymph nodes and spleen and a persistence of the thymus have been described

The changes which occur in lymphocytes and lymphoid tissues have received many interpretations It has been attributed to a constitutional abnormality not directly due to the thyroid [35] A direct stimulating effect of the thyroid hormone on lymphoid tissue has been suggested [36] Menkin[37] believed that the relative lymphocytosis in cases of exophthalmic goiter was due to sympathetic stimulation of lymphoid structures particularly the spleen

All authors do not agree that the lymphocytes are significantly increased Hertz and Lerman [38] using supravital staining technics concluded that the most marked and characteristic finding in the blood of patients with exophthalmic goiter was a relative and absolute monocytosis The lymphocytes were found to be normal in absolute numbers The discrepancy of their observations as compared with the findings of others was attributed to the greater accuracy of the staining technic that they used

In recent years the influence of adrenal hormones on lymphocytes and lymphoid tissue has been emphasized (vide infra) It is now evident that certain adrenal steroids oxygenated on the eleventh carbon atom cause a decrease in the number of circulating lymphocytes and a decrease in size of the thymus and lymph nodes Selye[39] has described an apparent antagonism between the thyroid hormone and adrenal hormones in controlling the size of the thymus No significant decrease in the weight of the thymus of rats occurred after thyroidectomy suggesting that this hormone is not requisite for the persistence of the thymus However the induction of stress in such rats by means of formaldehyde injections was followed by a severe and often fatal alarm reaction The involution of lymphatic organs and thymus was much more marked than in intact animals It appears therefore that animals which have been thyroidectomized are greatly sensitized to the action of adrenal hormones

From these observations it would seem reasonable to suppose that the lymphocytosis and persistence of thymus which has been described in thyrotoxicosis

propionate was administered three times a week in a dose of 25 mg by intramuscular injection Ferrous sulfate 0 75 Gm per day was given for the last two weeks of her hospital stay A progressive improvement of red blood cell count from 3 9 to 4 7 million occurred and the hemoglobin concentration increased from 65 per cent to 75 per cent The same therapy was continued after discharge from the hospital and examination of the blood on January 28 1944 showed normal values

Comment This woman noted the onset of symptoms of hypometabolism following delivery However there was no abnormal bleeding or shock and the history of normal lactation and the reappearance of regular menstrual cycles indicates that if postpartum necrosis of the pituitary was the cause of her hypothyroidism there was no *pan*hypopituitarism However the laboratory data are consistent with primary thyroid myxedema She presented a moderately severe normocytic hypochromic anemia which did not respond to therapy with vitamins liver extract and iron The hemoglobin had risen slightly after five months of treatment with desiccated thyroid The addition of testosterone propionate by intramuscular injection seemed to accelerate blood regeneration The combination of iron testosterone propionate and thyroid proved effective in restoring the blood to normal

The changes in the blood in hyperthyroidism have received much study The early clinicians believed that anemia was an important feature of the disease and in particular emphasized the association of hyperthyroidism with so called chlorosis With improvement in methods for counting red blood cells and estimating hemoglobin and the systematic application of these methods to large groups of patients it became apparent that some of the features of thyrotoxicosis which had suggested anemia had been misinterpreted In the first comprehensive study of the blood in this disease by Kocher [31] the red blood cell count was generally normal and sometimes even above the levels then accepted as normal Subsequent work by many authors has sustained this finding

Changes which occur in the white cells have given rise to a great deal of study and speculation After examining the blood of 106 patients with thyrotoxicosis Kocher[31] described in 1908 what he believed to be the pathognomonic blood picture in this disease He emphasized the following features a tendency toward leukopenia with a reduction mainly of polymorphonuclear neutrophils both a relative and absolute increase in lymphocytes a moderate increase in eosinophilic leukocytes The changes in the white blood cells were claimed to be a reliable index of prognosis and the return to normal was believed to be a valuable indication of successful treatment

Most subsequent investigators have been unable to confirm the significance of many of the features of the Kocher blood picture Leukopenia was found to be usually slight and frequently absent In general most of the reports have confirmed the tendency toward lymphocytosis but the diagnostic usefulness of this change was lost when many other causes of lymphocytosis were recognized

Recently Bistrom[3] has carefully reinvestigated this problem He made a comparative study of the morphology of the peripheral blood and bone marrow in patients with nontoxic goiters toxic goiters and in control subjects admitted to the hospital for minor surgical procedures The peripheral blood and bone marrow of patients with nontoxic goiters did not differ significantly from the control group

White and Dougherty[51] have studied the effects of adrenotrophic hormone and adrenal cortical steroids on red cells and hemoglobin A single injection of either substance will cause a transitory increase in the red blood cell count followed by a decrease to lower than pretreatment levels The continued injection of adreno trophic hormone resulted in a significant increase in red blood cell count and hemoglobin The authors concluded that it is possible that repeated hormone injection eventually leads to a stimulated production of red cells in an effort to compensate for the diminution of erythrocyte count produced by a single dose of hormone

In a number of experimental conditions the size of the thymus varies inversely with the activity of the adrenal cortex This has been most clearly demonstrated in the response of an animal to stress Selye[39] has introduced the concept of the alarm reaction as a phase in the general adaptation of the body to harmful stimuli An increase in the size and activity of the adrenal cortex is a fundamental element in this process One of the most striking and constant changes is the invo lution of the lymphatic organs The loss of weight is most marked and rapid in the thymus Here the characteristic cells of the parenchyma the thymocytes actually disintegrate and twenty four hours after the onset of a severe alarm re action only the debris of their chromatin is left lying partly free in the reticulum partly in phagocytes which are engaged in removing it The lymph nodes also show signs of involution but without any noticeable hyperplasia of the reticulum The involution in them usually begins in the germ centers which may disappear almost completely That the involution of the thymus is secondary to the adrenal cortical activity seems probable because no change occurs in adrenalectomized animals The injection of certain adrenal steroids into intact animals or adrena lectomized animals and th injection of adrenocorticotrophic hormone into intact or hypophysectomized but not adrenalectomized animals will reproduce similar changes in lymphoid tissut and thymus[6, 53]

Dougherty and White[7] e[54] have confirmed and extended these observations by re porting a remarkable decrease in the circulating lymphocytes after the injection of adrenocorticotrophic hormone or cortical steroids into the mouse rat rabbit and dog This phenomenon has been termed lympholysis Lympholysis in the mouse is particularly striking Within an hour after the injection of adrenocorticotro phic hormone there occurs a definite fall in the circulating lymphocytes which reaches a maximum in six to nine hours Recovery to normal takes place within twenty four hours Probably considerable variation in sensitivity of lymphocytes to lysis exists because in our laboratory[55] rats of the Sprague Dawley strain have shown little lymphopenia after injection of aqueous adrenal cortical extract

Minor changes in chemical structure have been shown to modify the action of various adrenal steroids on lymphocytes 11 Desoxycorticosterone has been demon strated to be without effect[2] On the other hand Compound E (11-dehydro 17 hydroxy corticosterone) and other active steroids with an oxygen on the eleventh carbon atom possess this property

The destruction of lymphoid tissue is believed to be the source of the increase in

might be the expression of a relative deficiency of adrenal steroids Some evidence that such a deficiency exists has been obtained in this laboratory [40] Despite the severe stress imposed on patients with thyrotoxicosis the excretion of cortin as measured by a chemical method is frequently markedly subnormal This observation has been interpreted tentatively as indicative of increased breakdown of hormone although more studies are necessary to establish this as a fact

3 The Adrenal Cortex

Great advances have been made in the understanding of the physiology and chemistry of the adrenal cortex The adrenal secretes several substances which have unrelated and even antagonistic actions One of the most interesting phases of adrenal physiology has been the demonstration of the influence of the adrenal on lymphocytes and the release of antibodies

In all laboratory animals adrenalectomy results in death after a short period if replacement of salt and/or hormones is not provided Adrenalectomized rats show an increase in polymorphonuclear leukocytes and lymphocytes about twenty four hours before death [4] An increase in the weight of the thymus and systemic lymph nodes has been reported in adrenalectomized rats maintained in good condition by means of the addition of sodium chloride to their drinking water [4] In cats surviving a relatively long time after operation there is a decrease in polymorphonuclear cells and an increase in lymphocytes [43] Increases which have been reported in the red blood cell count and hemoglobin concentration are indications of the disturbed electrolyte and fluid metabolism which causes marked hemoconcentration [44] If adrenalectomized cats and dogs are maintained in fair general condition by several different methods of treatment the peripheral blood is essentially normal

Adrenal insufficiency in human beings produces changes similar to those in experimental animals During an adrenal crisis there is usually a slight increase in red blood cell count and hemoglobin and a relative lymphocytosis Restoration of normal blood volume by the use of desoxycorticosterone and saline solution frequently reveals a mild underlying anemia A relative lymphocytosis may persist even after treatment [46]

Polycythemia has been a frequent but not a constant finding in Cushing s syndrome [47 48] Approximately one half of the well authenticated cases have shown an elevation of the red blood cell count [49] Gunther[50] has collected data on 7 patients with hyperadrenalcorticism with red counts in excess of six million The abnormality is usually mild and associated mainly with rapid progress of the disease In itself it does not seem to produce the complications which are observed in polycythemia vera Many patients with Cushing s syndrome are suspected of having polycythemia in whom subsequent blood counts fail to confirm the clinical impression A plethoric appearance of the cheeks is common and is due to atrophy and stretching of the skin which permits the transmission of the color of the under lying venous plexuses Combined with the marked deposition of fat about the face and neck the red cheeks constitute the pathognomonic facies described by Cushing

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serum beta and gamma globulins which follows injections of active hormones of the adrenal cortex Direct analysis of lysed lymphoid tissue washed free of all blood serum has demonstrated proteins which are indistinguishable from serum beta and gamma globulins [56] In immunized animals the lymphocyte contains antibodies which are liberated into the blood stream by the process of lympholysis [57] Dougherty and White[54] believed that the above mechanism is the explanation for the anamnestic reaction which occurs in acute infection

The effect of adrenal steroids on the lymphocytes of human beings has not been reported in any detail Dougherty and White reported observations on several patients with lymphocytic leukemia and also in control subjects A suggestive drop in the lymphocytes was observed in some of the patients Forsham et al [58] observed the effects induced by synthetic 11 dehydrocorticosterone acetate on the lymphocytes In thirteen experiments on patients with Addison s disease 20-60 mg of the compound were given as a daily dose No significant decrease in the lymphocytes was found It was noted however that there was an increase in urinary uric acid excretion in comparison to creatinine These data seem to suggest that a tissue rich in nucleoproteins was being broken down and it is therefore possible that there was significant lympholysis which was not manifest in the circulating blood Perera at al [59] have used the same compound and found an inconstant decrease in the lymphocytes following administration to human beings Recently Thorn s collaborators[60] have presented interesting observations on the effect of adrenocorticotropic hormone on the blood picture of man In normal subjects four hours after the administration of 25 mg of adrenocorticotropic hormone by intramuscular injection there was a 90 per cent increase in the absolute number of polymorphonuclear neutrophils a 40 per cent decrease in lymphocytes and a 78 per cent decrease in eosinophils Similar changes did not occur in patients with Addison s disease However the administration of 20 mg of 17 hydroxycorticosterone to such patients caused an increase of 129 per cent in the absolute number of polymorphonuclear neutrophils a decrease of 53 per cent in the lymphocytes and a decrease of 76 per cent in the eosinophils

In our experience little change in the relative or absolute number of lymphocytes follows the administration of adrenal cortical extract in doses of from 10 to 50 cc to patients with adrenal insufficiency Figure 2 shows the results which we have obtained on treating a patient with hypopituitarism for nine days Both adrenal cortical extract (Upjohn) and lipoadrenal cortical extract (Upjohn) were given in doses which are in excess of tho e required to maintain patients with Addison s disease

Because of the apparent failure of moderate doses of adrenal cortical extract to alter the blood picture larger doses were administered A patient with hypopituitarism secondary to a chromophobe adenoma was selected Immunization against heat killed typhoid bacilli was achieved by three injections of 0 1 cc of a standard vaccine * It is interesting that despite the small size of the immunizing

* This material was very kindly suppl ed by the Department of Public Health Antitoxin and Va cine Laboratory of the Commonwealth of Ma achusetts

dose the patient noted marked local and systemic symptoms following each injection and that there was a satisfactory rise in antibody titer Forty cc of adrenal cortical extract was given in two equal doses by intramuscular injection Figure 3 shows the changes which occurred in the lymphocyte count the anti typhoid agglutination titer and the titer of anti B isoagglutinin (the patient was blood group A) A brisk fall in the number of lymphocytes was noted after six hours with a return to normal after twenty four hours A rise in the antityphoid agglutination titer was noted at six hours there was a decline by twenty four hours and a return

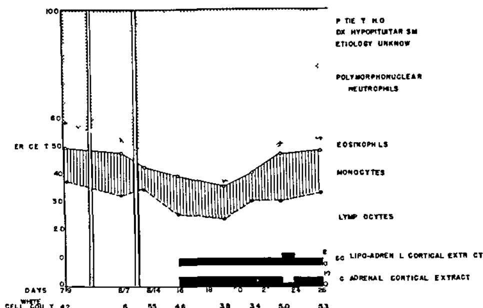


FIG 2 CHANGES IN DIFFERENTIAL WHITE BLOOD CELL COUNTS IN A PATIENT WITH SIMMOND DISEASE AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACTS

Note the apparent drop in eosinophils with negligible changes in other cell element

to the former titer by six days No significant change occurred in the anti B isoagglutinin titer

The experiment was repeated after the injection of 50 mg of testosterone propionate daily for three days and 25 mg for four days Following this treatment the antityphoid titer seemed to decline The changes which followed injection of adrenal cortical extract were similar to those observed in this patient prior to testosterone therapy

We have concluded from these limited observations that the lowering of the lymphocyte counts with a rise in immune bodies which has been observed in laboratory animals can be reproduced in human beings but that relatively large doses of adrenal cortical extract are required

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a similar manner Destruction of the pituitary in man frequently results in a moderate anemia

The decrease in the red blood cell count and hemoglobin which occurs in the hypophysectomized rat has received the most intensive study In 1935 Stewart Greep and Meyer[64] noted that hypophysectomized rats were anemic and that there was a decrease in the number of reticulocytes A rise in reticulocytes occurred in normal rats exposed to reduced oxygen tension but not in hypophysectomized ones Increases in the red blood cell count and hemoglobin of the hypophysectomized rats occurred only if the stimulus of low oxygen tension was applied soon after operation The reticulocyte response following substitution therapy parenterally has proved an unreliable criterion because reticulocytosis has been produced by many hormonal and nonhormonal agents [65] In general reticulocytosis has been unaccompanied by improvement in red blood cell count and hemoglobin concentration The conclusion was reached that the alterations in the blood following hypophysectomy were due to general disturbances in metabolism rather than the absence of a specific hematopoietic hormone

On the other hand the existence of a specific pituitary hormone which stimulates blood formation has been postulated by Moehlig and Bates [66] The polycythemia of Cushing s disease was attributed to an excessive production of this factor

On the basis of the response of hypophysectomized rats to oral administration of preparations of anterior pituitary Flaks Himmel and Zlotnik[1] [67] postulated the existence of an erythrogenic hormone They claimed that the anemia of hypophysectomized rats was repaired and that polycythemia was produced by their hormone preparation in intact animals It is difficult to accept this claim because of the impotency of known pituitary hormones when administered orally

Beneficial effects on the anemia of hypophysectomized rats have followed treatment with several hormones pituitary as well as non pituitary Vollmer and Gordon[68] and Vollmer Gordon and Charipper [69] found that testosterone propionate increased the red blood cell count of hypophysectomized male and female rats Estradiol on the other hand seemed to intensify the anemia Pregnant mare s serum raised the red blood cell count of hypophysectomized male rats but lowered the counts in hypophysectomized female rats Thyroxine plus testosterone was moderately effective Prolactin eemed to be inactive Desoxycorticosterone produced no definite effect on the bone marrow

Various types of replacement therapy have been investigated by Crafts [70] [71] In *hypophysectomized female rats the anemia was found to be microcytic and hypo*chromic and accompanied by hypoplastic changes in the bone marrow Iron or iron plus copper delayed slightly the onset of anemia Injection of 0 01 mg of thyroxine daily maintained a normal erythrocyte count but did not prevent a decrease in hemoglobin concentration Histologic examination revealed greater cellularity of the bone marrow and decreased infiltration by fat cells This was interpreted as an indication of increased activity The combination of thyroxine iron and copper maintained a normal red cell count and seemed to increase the amount of hemoglobin Hypophysecromized adult male rats developed a severe microcytic hypochromic anemia with hypoplastic changes in the bone marrow Testosterone

Other mechanisms of regulation of lymphocytes than pituitary adrenal control must exist This is evident from the study of adrenalectomized rats maintained on salt Crafts[17] found that such animals maintained a normal white count and differential count De La Balze Reifenstein and Albright[48] reviewed their extensive experience with adrenal disorders in man and could not establish a significant increase in the absolute number of lymphocytes in patients with Addison s disease although the relative number of lymphocytes was increased

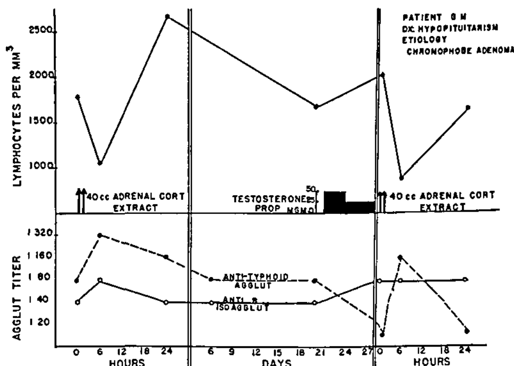


FIG 3 NOTE THE LYMPHOPENIA AND INCREASE IN THE TITER OF ANTITYPHOID AGGLUTINATION FOLLOWING THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT

4 THE PITUITARY GLAND

Discussion of the possible mechanisms by which this gland alters hematopoiesis has been reserved until last It is well known that the anterior lobe of the pituitary gland controls the activity of the other endocrine glands and it could thereby aid in the regulation of formed elements in the blood It is therefore evident that the anemia which is often observed following destruction of the anterior pituitary is a complicated phenomenon

An anemia following hypophysectomy was first noted by Aschner[61] in dogs in 1912 Later it was determined that rabbits[6] and rats [63] respond to the operation in

particularly valuable for an understanding of the anemia in Simmonds disease because the time of onset can be fixed with some certainty and it occurs in young adult females who may live with hypopituitarism for many years. Quite frequently the blood deficit of the precipitating hemorrhage is incompletely restored. However, for the first five years it is not uncommon for these patients to maintain relatively normal red blood cell counts but with a rather low hemoglobin concentration. During the second five year period there is a definite tendency for the red blood cell count to decrease to between 3 and 4 million with some increase in the color index. The blood frequently remains at this level indefinitely. Occasionally there is a further decrease in the red blood cell count to a level of from 2 to 3 million with a color index of 0.95 to 1.25. These cases showing a severe anemia and a tendency toward macrocytosis seem to occur in patients with more profound evidences of deficient thyroid function and may be indistinguishable clinically from thyroid myxedema. Leukocytes usually number between 4 to 6 thousand although leukopenia is common. The smear characteristically shows a relative lymphocytosis with a moderate eosinophilia in about two thirds of the cases.

The results of therapy on the blood in hypopituitarism have received scant attention in the literature. The use of anterior pituitary hormones is the rational approach to the problem but has not proved successful. Extracts of the pituitary of high potency and purity have not been available. Because of their protein nature pituitary extracts rapidly lose their effectiveness because of the development of antihormones. Also allergic manifestations such as urticaria and local pain are common. Because of these disadvantages it has been more practical to use the hormones of the atrophic end-organ glands.[9, 10] The combination of desiccated thyroid, desoxycorticosterone acetate and testosterone has provided a reasonably satisfactory method of treatment.

In this clinic the above therapy has been used with various modifications in the treatment of Simmonds disease. Marked relief from many of the disabling symptoms of this disease has been obtained but improvement in the blood findings has been inconstant. The following case (case 2) is reported in detail because this patient had been under observation for a period of nine years and during this period adequate trial with many therapeutic agents failed to correct an anemia. Case 3 is reported to show that improvement in the blood may follow hormone treatment without the addition of liver or iron. Hematologic data on 22 patients with hypopituitarism observed during the past six years are presented in table 1.

Pituitary fibrosis of unknown etiology associated with a moderately severe normocytic and normochromic anemia which failed to respond to liver, iron and hormone therapy

Case 2. F. S. (Details of this case have been reported elsewhere.[29, 30]) A white housewife of American parentage, aged 38, entered the Boston City Hospital October 18, 1934 complaining of weakness and vomiting. Following an attack of influenza two years prior to entry she had been told that she had anemia and low blood pressure. Recovery from this illness was protracted and incomplete and she continued to suffer from asthenia, fatigability, drowsiness and anorexia. A local physician prescribed ground raw liver which she took for a period of about six months without improvement in any of her complaints. She sought hospital care after six weeks of nausea and vomiting. Her past history revealed that she had given birth to a normal child about ten years previous to entry. A hysterectomy was performed some time after delivery for pelvic peritonitis. Unfortunately details of the delivery and operation are not available.

therapy prevented the decrease in the red blood cell count and restored the bone marrow to normal cellularity Microcytosis and hypochromia were only partially corrected Because the anemia following castration was much less marked than that following hypophysectomy, the author believed that the anemia in the latter condition was not a manifestation of decreased androgens although androgens proved partially effective in preventing the experimentally induced anemia

An anemia following destruction of the anterior lobe of the pituitary in man has been long recognized as a significant feature of the clinical syndrome called Simmonds disease Silver[7] reviewed the literature in 1933 and observed that anemia is a constant finding The hemoglobin averages 50 per cent with a color index which is usually less than one Leukopenia is common and an eosinophilia reaching 22 per cent may occur

Snapper Groen Hunter and Witts [3] described 6 cases which they believed presented evidences of pituitary or gonadal deficiency plus an anemia Case 2 of their series was a woman with pituitary necrosis following hemorrhage and shock at the time of delivery A macrocytic anemia of 3 2 million red blood cells was present Case 3 was a patient with hypopituitarism secondary to a chromophobe adenoma There was a moderately severe macrocytic anemia accompanied by gastric achylia and evidence of combined system disease In the other cases hypogonadism was probably primary The importance of achlorhydria and defective absorption was stressed as the immediate cause of anemia leading to deficiency of either iron or the hemopoietic principle of liver extract Witts[74] later described two additional cases which he considered to be examples of hypopituitarism and which were associated with a macrocytic anemia responding to liver He concluded The association of pernicious anemia with hyperthyroidism with pregnancy and with pituitary disease suggests that there is a hormonal element or mechanism which can lead to the degeneration of the cells which secrete intrinsic factor We may consider the association of pernicious anemia with hypopituitarism as another example of the precocious senile changes to which the patient with pituitary disease is liable

The effect of testosterone propionate on the anemia of hypopituitarism has been the subject of a recent report [75] Two cases of hypopituitarism with anemia are described that responded to the combined administration of testosterone and liver In one patient at least it is evident that no response occurred with liver extract alone It was suggested that testosterone enabled the bone marrow to utilize the hematinic principle which it previously had been unable to do

Escamilla et al [76] have reviewed 101 cases of Simmonds disease verified by post mortem examination Among this group the hemoglobin ranged from 102 per cent to 40 per cent with an average of 65 per cent Red blood cell counts ranged from 5 6 to 2 0 averaging 3 7 million Eosinophilia was commonly observed with 6 3 per cent as the average figure

Sheehan[77, 78] has emphasized the importance of serious hemorrhage and shock at the time of delivery as an etiologic factor producing necrosis of the anterior lobe of the pituitary The lesion is a thrombotic infarction of variable extent which later may reduce the anterior lobe to a nubbin of fibrous tissue This group of cases is

particularly valuable for an understanding of the anemia in Simmonds disease because the time of onset can be fixed with some certainty and it occurs in young adult females who may live with hypopituitarism for many years Quite frequently the blood deficit of the precipitating hemorrhage is incompletely restored However for the first five years it is not uncommon for these patients to maintain relatively normal red blood cell counts but with a rather low hemoglobin concentration During the second five year period there is a definite tendency for the red blood cell count to decrease to between 3 and 4 million with some increase in the color index The blood frequently remains at this level indefinitely Occasionally there is a further decrease in the red blood cell count to a level of from 2 to 3 million with a color index of 0 95 to 1 25 These cases showing a severe anemia and a tendency toward macrocytosis seem to occur in patients with more profound evidences of deficient thyroid function and may be indistinguishable clinically from thyroid myxedema Leukocytes usually number between 4 to 6 thousand although leukopenia is common The smear characteristically shows a relative lymphocytosis with a moderate eosinophilia in about two thirds of the cases

The results of therapy on the blood in hypopituitarism have received scant attention in the literature The use of anterior pituitary hormones is the rational approach to the problem but has not proved successful Extracts of the pituitary of high potency and purity have not been available Because of their protein nature pituitary extracts rapidly lose their effectiveness because of the development of antihormones Also allergic manifestations such as urticaria and local pain are common Because of these disadvantages it has been more practical to use the hormones of the atrophic end organ glands[79, 80] The combination of desiccated thyroid desoxycorticosterone acetate and testosterone has provided a reasonably satisfactory method of treatment

In this clinic the above therapy has been used with various modifications in the treatment of Simmonds disease Marked relief from many of the disabling symptoms of this disease has been obtained but improvement in the blood findings has been inconstant The following case (case 2) is reported in detail because this patient had been under observation for a period of nine years and during this period adequate trial with many therapeutic agents failed to correct an anemia Case 3 is reported to show that improvement in the blood may follow hormone treatment without the addition of liver or iron Hematologic data on 22 patients with hypopituitarism observed during the past six years are presented in table 1

P tu t y fib osi of nk un ti logy a sat d ith a moderately severe norm cytic and normochrom c a mia ub h fa l d t resp nd to li r i on and hormon th apy

Ca 2 F S (Deta l of this case have been reported elsewhere[79, 8]) A white house ife of American p entage aged 38 entered the Boston City Ho pital October 18 1934 complaining of weakness nd vomiting Following an attack of influenza two yea s prior to entry she h d been told that she had anemia and low blood pressure Reco ery from this illness was protracted and incomplete and she continued to s ffer from asthenia f tigabil ty dro siness nd a orexia A local physic an pre cribed ground raw li er which he took for a period of about six months without impro ement in any of her compla nts She sought ho pital care after *su* weeks of nausea and omiting Her p st history revealed th t she had g en birth to a normal child about ten years previous to entry A hysterectomy was performed some time after delivery for pelvic peritonitis Unfo tunately det ls of the delivery and operation are not available

On physical examination she appeared to be an underdeveloped and poorly nourished woman The skin was pale smooth and had a yellowish waxy texture The tongue had normal papillae The blood pressure was 9_ mm of Hg systolic and 68 diastolic A mass interpreted as the liver edge was felt on

TABLE 1 —*Hematologic Observations in Hypopituitarism*

All cases presented clinical and laboratory evidence indicating deficiency of two or more of the following hormones gonadotropic thyrotropic adrenotropic and growth hormone

PART I POSTPARTUM NECROSIS OF THE PITUITARY

Case	Duration of D s e Yea s	Age	Date	% Hgb 100% = 15 6 Gm	Eryth ocytes × 10⁶/mm³	% Reticulocytes	M C V cu mi c s	M C H m cro m crogr ms	M C H Conc %	Leukocytes × 10 /mm	Tot l Gr ulo cyt s %	Eos noph ls %	Lymph cyt %	M cyt s %
T R	3	21	4/29/46	68	3 6	0 8	90	29	33	8 6	69	3	21	10
			Given desiccated thyroid 96 mg/day One pellet (128 mg) DOCA implanted Testosterone propionate 25 mg intramuscularly daily for 17 days											
			6/18/46	70	4 1	3 8	87	27	31	4 8				
			Desiccated thyroid continued 10 mg of methyl testosterone daily for one month Stilbestrol 0 4 mg daily for the next month Later given 3 courses of Dienestrol for 21 days followed by Progestoral for five days											
			4/15/47	83	4 6		88	28	32	8 0				
C R	4	23	2/28/47	87	4 5	0 8	93	31	33	6 1	54	0	33	13
A N	12	39	11/ 9/42	61	2 8*					8 9*	75*	1*	25*	0*
H O N	14	42	5/28/41	71	3 5	0 2	87	32	37	2 1	72 5	2	20	7 5
			1/17/42	55	2 5	2 0	111	34	31	3 0	62 5	0 5	29 5	8
M T	20	61	9/ 5/45	55	3 3	1 0	89	25	29	3 1	37 5	3	37 5	34 5
			9/ 6/45	54	3 1	1 0	92	27	30	3 4				
			Desiccated thyroid 64 mg/day Three pellets (each 75 mg) of testosterone implanted											
			9/25/45	52	3 1	1 0	94	26	27	3 6				

CLINICAL ABSTRACT *T R* F Severe postpartum hemorrhage shock (1943) Failure of lactation fatigue and symptoms of hypometabolism FSH absent 17 KS 4 4 *C R* F Severe postpartum hemorrhage and shock (1943) Failure of lactation Amenorrhea for 2 years then very infrequent menses Symptoms of hypometabolism BMR —31 Water test positive *A N* F Severe postpartum hemorrhage and shock (1930) Pituitary fibrosis found on post mortem examination *H O N* F (see case 3 in text) *M T* F Onset followed delivery of twins (1925) Fibrosis of pituitary found on post mortem examination

Determinations carried out by ward laboratories

deep inspiration at the right costal margin The spleen could not be felt and there was no lymphadenopathy The only abnormal neurologic finding as a questionably positi e Babinski sign bilaterally

Laboratory examinations sho ed the follo ving The concentration of hemoglobin was 63 per cent and there were 3 35 million red blood cell The hite blood cell count was 4 800 with 66 per cent polymorphonuclear neutrophils and 33 per cent lymphocytes The hematocrit as 31 2 p r cent Urinalysis was not abnormal The Kahn test as at first reported as doubtful but subsequent tests vere nega

tive No occult blood was present in the stools Free acid was not present in the gastric juice even after the administration of histamine The icteric index was 6 units The serum cholesterol was 137 mg per

TABLE 1 PART II PITUITARY FIBROSIS OF UNKNOWN CAUSE

Case	Duration of Disease, years	Age	Date	% Hgb 100% = 15.6 Gm	Erythrocytes X 10⁶/mm	% Reticulocytes	MCV cu microns	MCH micromicrograms	MCHC %	Leukocytes X 10³/mm	Total Granulocytes %	Eosinophils %	Lymphocytes %	Monocytes %
F S	2	38	1/22/35	70	3 9	0 4	82	28	34	7 8	73	1	24	3
			9/23/36	77	3 6		89	34	37		60 5	3 5	30 5	9
			1/ 2/41	68†	3 6†		86†	30†	35†	5 4†				
H O	?5	53	7/19/46	66	2 9	0 8	111	33	33	4 2	55	9	30	15
			DOCA 2 mg intramuscularly daily Testosterone propionate 25 mg intramuscularly daily for 11 days thereafter every other day Reticulocytes increased to a maximum of 5 6% on 7/28/46											
			8/14/46	68	3 1	2 0	98	34	35	5 5	57	4	35	8
			Three 75 mg pellets of testosterone and one 75 mg pellet of DOCA implanted subcutaneously											
			3/ 6/47	78	3 7		95	33	35	5 2				
A B	?9	39	7/21/45	70	3 0					7 0	51	1*	49	0*
			Desiccated thyroid 32 mg daily Implantation of three 75 mg pellets of testosterone and one 75 mg pellet of DOCA											
			5/15/47	69	3 5		92	31	33	8 1	60	4	33	7
E J	?	68	7/31/46	68	3 3*					5 6*	90	1	5	5
S K	?	69	1/25/46	77*	3 0					6 1	69	0*	29	2
			Desiccated thyroid by mouth and DOCA and testosterone propionate by intramuscular injection											
			2/ /47	82	4 0									
A A	?	61	9/28/46	61	3 4		93	27 3	30					
			Desiccated thyroid given in small increasing doses											
			10/21/46	50	2 5		101	32	31	4 9	44	11	61	3
			Desiccated thyroid testosterone propionate liver extract (Lilly) 2500 cc of whole blood											
				79	4 5	0	91	27	30					

CLINICAL ABSTRACT *F S* F Pituitary fibrosis found at post mortem examination (see case 2 in text) *H O* F Intrasellar calcification FSH absent 1 KS 1 0 mg/day Insulin sensitive *A B* F Insulin sensitive FSH negative Decreased cortin excretion *E J* F Pituitary fibrosis at post mortem *S K* M Marked insulin sensitivity FSH absent Decreased cortin excretion *A A* F Marked insulin sensitivity FSH absent 17 KS 3 3 mg/day

Determinations carried out by ward laboratories

† Determinations carried out by Hematology Laboratories Mass Memorial Hospital Dr Chester Keefer has kindly consented to the inclusion of these data

100 cc A glucose tolerance test was within normal limits The basal metabolic rate was −26 per cent X ray examination of the chest upper and lower gastrointestinal tract and sella turcica were within normal limits

TABLE 1 PART III HYPOPITUITARISM SECONDARY TO NEOPLASMS

Case	Duration of Disease Years	Age	Date	% Hgb 100 = 15 6 Gm	Erythrocytes $\times 10^6$/mm^3	% Reticulocytes	MCV cu microns	MCH micromicrograms	MCH Conc %	Leukocytes $\times 10^3$ mm	Total Granulocytes %	Eosinophils %	Lymphocytes %	Monocytes %
S B	0 5	58	6/22/38	75*	3 5*					4 2*	65	0	32*	3*
			3/29/45	61	3 8	0 8	88	25	29	5 5	53 5	1 5	34 5	12
M R	1	34		75						5 1*				
G M	2	42	2/21/44	59	3 6		88	26	29	6 5	68 5	3	19 5	12
			Received 5 cc of liver extract (Reticulogen Lilly) in three injections which was followed by no significant increase in reticulocytes Testosterone propionate 25 mg intramuscularly injected three times a week											
			4/18/44	88	4 4	0 5	89	31	35					
D N	5	38	3/ 7/42	69	3 2		99	25	30	4 4				
			No reticulocytosis following three daily injections of 10 USP units of liver extract (Reticulogen Lilly) One pellet (150 mg) of testosterone and six (125 mg) pellets of DOCA implanted Detailed metabolic studies are described elsewhere[78]											
			3/31/42	85	4 1		89	37	32	6 8				
P S	6	46	3/29/43	95	4 8		91	31						
			Had received replacement therapy for about two years[78]											
J C	16	59	8/ 1/46	70*	4 2					6 9	60		40	
H A	?	66	7/ 5/44	67	4 0					6 2	72*		24	4
C B	1	43	1/29/46		5 4*					10 5*				
J D	?5	20	5/ 3/44	81						7 5				
J B	?9	37	8/12/43	76	4 9					9 4				
M H	?6	13	1/20 42	80	4 2					12 9				

CLINICAL ABSTRACT *S B* M Pituitary tumor *M R* M Pituitary tumor *G M* M Chromophobe adenoma ‡ *D N* F Pituitary tumor *P S* M Chromophobe adenoma *J C* M Chromophobe adenoma ‡ *H A* M Chromophobe adenoma complicated by annular carcinoma of transverse colon found on post mortem examination *C B* M Pituitary tumor *J D* M Suprasellar cyst with mild hypopituitarism ‡ *J B* M Suprasellar cyst with moderately severe hypopituitarism *M H* F Apolar neuroblastoma involving pituitary stalk with hypogonadism and dwarfism BMR normal FSH absent

* Determinations carried out by outward laboratories

‡ Diagnosis established at operation

A high protein diet with vitamin B complex was given In addition she received iron liver and desiccated thyroid separately and in combination for intervals of several weeks without a reticulocyte response

or improvement in her anemia (see figure 4) Removal of some badly infected teeth seemed to improve her general condition The patient was discharged from the hospital in April 1935

She was seen by Dr Juda Groen in September 1936 at the Boston City Hospital at which time she reported slight improvement in appetite and strength although she had failed to take d siccated thyroid regularly Signs of myxedema were evident and the absence of axillary and pubic hair was recorded The blood findings were much the same as before

At this time the patient failed to return for further treatment and continued to live a restricted life at home Fatigue and somnolence became increasingly incapacitating until by 1941 she was almost bed ridden Anorexia became almost total and in No ember 1941 she had an episode of confusion and dis-

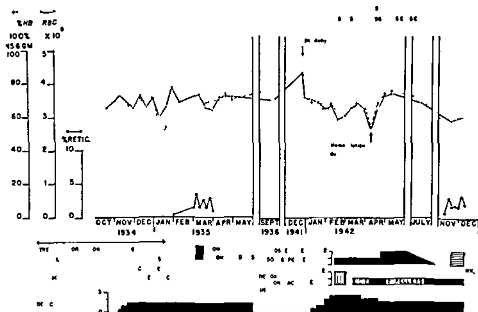


Fig 4 Changes in Red Blood Cell Count and Hemoglobin Concentration in Case 2

Data have been plotted as averages for ten day periods Note the apparent absence of response to many different forms of treatment

orientat on and because of this she was sent to the Massachusetts Memorial Hospital She as found to be acutely dehydrated and in poor cond tion on admission Numerous petechiae were present o er the right arm The blood pressure was recorded as 108 mm of Hg systolic and ,8 diastolic After adequate rep ir of her dehydration it was evident th t her anemi was practically identical to that observed seven years previously The red blood cell count as 3 78 million and the hemoglobin concentration was 65 per cent The hite blood cell count on admission was 6 400 with 43 per cent lymphocytes present The platelets numbered 172 000 Aspiration of bone marrow wa performed and the following cell distribution was found

Polymorphonuclear leukocytes	3 per cent
Band forms	12 per cent
Myelocytes	30 per cent
Myelobla ts	6 per cent
N cleated red blood cells	26 per cent
Erythroblasts	8 per cent
Stem cells	3 per cent
Lymphocyte	9 per cent
Monocytes	3 per cent

No follicle stimulating hormone was found in the urine upon testing for 10 rat units per twenty four hours The patient excreted 0 2 mg of 17 ketosteroids per twenty four hours An insulin tolerance test using 0 05 units of insulin per kilogram of body weight intravenously demonstrated moderate hypo glycemic unresponsiveness A water test[29] was positive but a salt deprivation test[30] was negative The basal metabolic rate was −23 per cent

On January 9 treatment was begun with desiccated thyroid 32 mg daily and this was progressively increased to 160 mg daily Two pellets of desoxycorticosterone acetate 125 mg each and two pellets of testosterone 150 mg each were implanted subcutaneously The patient was much improved by this treatment as far as her general condition was concerned but no improvement in her anemia occurred and she was discharged from the hospital in May 1942

In November 1943 she entered the Boston City Hospital for replenishment of her therapy She was given ferrous sulfate by mouth and desoxycorticosterone acetate and testosterone propionate by intra muscular injection Therapy with desiccated thyroid was continued Unfortunately after a month in the hospital she developed virus pneumonia and died after a brief stormy course

At postmortem examination the pituitary was very small and fibrotic Microscopic study showed only a few acidophilic basophilic and chromophobe cells scattered in dense scar tissue The thyroid gland was one half normal size and the acini were very atrophic Extensive adrenal atrophy and fibrosis was found Microscopic examination of the bone marrow was interpreted as showing less than normal erythropoiesis There was no evidence of a defect in maturation Cells of the granulocytic series appeared to be normal except for a questionable increase in the eosinophilic series Megacaryocytes were of the usual number

Comment The etiology of the pituitary fibrosis in this case remains obscure The onset of symptoms following influenza suggests that this may have been the etiologic factor The anemia in this patient was normochromic and normocytic and remarkably constant in severity It was unaffected by raw liver by mouth liver by injection iron in several forms and by hormonal replacement treatment

Postpartum necrosis of the pituitary associated with a moderately severe normocytic and normochromic anemia with improvement after hormone therapy

Case 3 H O N (Details of this case have been reported elsewhere [79, 80]) A housewife aged 42 was admitted to the Boston City Hospital in May 1941 with the signs and symptoms of myxedema Twelve years prior to entry she had had a severe hemorrhage due to a placenta previa Following this episode menstruation never recurred and symptoms of hypometabolism were noted She was treated by several local physicians for hypothyroidism and anemia but the details are not available She took desiccated thyroid only at irregular interval and at the time of entry the classic features of myxedema were present with marked mental and physical retardation However her nutritional status was good Pubic and axillary hair was absent A hypotension of 90 mm Hg systolic and 50 diastolic was present The labora tory findings confirmed the diagnosis of Simmonds disease The basal metabolic rate was −43 per cent Plasma protein bound iodine was less than 1 microgram per cent Marked sensitivity and hypoglycemic unresponsiveness was demonstrated by an insulin tolerance test (0 04 units of insulin intravenously per kilogram of body weight) No follicle stimulating hormone was found in her urine when tested for 10 rat units per twenty four hours Free acid was not present in the gastric juice even after the administra tion of histamine

Hematologic data The red blood cell count was 3 45 million the hemoglobin was 71 per cent and the hematocrit was 30 per cent Calculation of the blood indices showed a mean corpuscular volume of 87 cu microns a mean corpuscular hemoglobin concentration of 37 per cent and a mean corpuscular hemoglobin of 32 micro-micrograms Two tenths of one per cent of all erythrocytes were reticulocytes The white blood cell count was 2 100 The differential count was as follows polymorphonuclear neu trophils 52 per cent band forms 14 per cent eosinophils 2 per cent basophils 4 5 per cent lymphocytes 19 per cent young lymphocytes 1 per cent monocytes 7 5 per cent

The patient received several pituitary hormones without clinical or hematologic improvement There was a decline of the red blood cell count and hemoglobin concentrations as shown in figure 5 Replacement therapy with nonpituitary hormones was begun on December 29 1942 with the adminis

tration of methyl testosterone 30 mg a day by mouth desiccated thyroid 30 mg a day by mouth and desoxycorticosterone acetate 2 mg a day by intramuscular injection At a later date pellets of testosterone and desoxycorticosterone acetate were implanted subcutaneously Details of therapy are indicated in figure 5

There was a remarkable improvement in the patient s general condition on this therapy Prior to treatment *the basal metabolic rate was −33 per cent and it rose to normal after treatment for 1 month* The myxedematous changes disappeared and there was a progressive improvement in her energy and strength The red blood cell count and hemoglobin concentration on January 17 1942 showed even lower values than previously The mean corpuscular volume on this occasion was 111 cu microns One month later the red blood cell count had increased to 4.05 million with a decrease in the mean corpuscular volume to normal size 83 cu microns and 4 5 per cent of the erythrocytes were reticulated On March

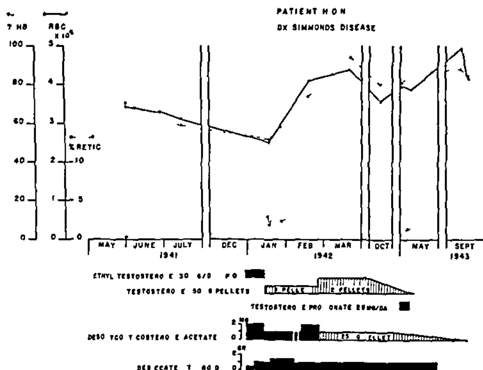


FIG 5 CHANGES IN RED BLOOD CELL COUNT AND HEMOGLOBIN CONCENTRATION FOLLOWING ENDOCRINE THERAPY IN CASE 3

20 1942 the red blood cell count and hemoglobin had returned to normal levels On follow up examination the improvement in the blood picture was largely sustained despite the fact that additional pellets of testosterone could not be procured for replacement

The patient re entered the Boston City Hospital in January 1944 with pneumonia and in spite of treatment with sulfathiazole adrenal cortical extract desoxycorticosterone and testosterone propionate she died four days after admission

At postmortem examination the pituitary was found to be very small and appeared on microscopic examination to be composed largely of fibrous tissue with rare nests of basophilic cells Marked atrophy of the thyroid was present and no tissue recognizable as adrenals was found On microscopic examination the vertebral marrow appeared hypoactive Red blood cell and white blood cell precursors were present and showed no evidence of a defect in maturation

No follicle stimulating hormone was found in the urine upon testing for 10 rat units per twenty four hours The patient excreted 0 2 mg of 17 ketosteroids per twenty four hours An insulin tolerance test using 0 05 units of insulin per kilogram of body weight intravenously demonstrated moderate hypo glycemic unresponsiveness A water test[29] was positive but a salt deprivation test[30] was negative The basal metabolic rate was—23 per cent

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Comment The onset of symptoms in this woman following a severe hemorrhage complicating a placenta previa strongly suggests that the atrophy and fibrosis of the pituitary observed at autopsy was due to postpartum necrosis A moderately severe normocytic anemia was present on admission and became more severe during the period of observation The macrocytosis observed in January 1942 may have been related to an increase in the severity of her thyroid deficiency as manifested by a basal metabolic rate of −53 per cent in December 1941 On combined hormonal treatment the red blood cell count and hemoglobin concentration returned almost to normal in the course of about three months It seems likely that testosterone propionate was the agent causing the improvement in the blood picture

Summary and Conclusions

Evidence has been presented that the gonads thyroid adrenal cortex and pituitary glands have a definite influence on blood formation The normal sex difference in erythrocyte levels in animals and probably in man can be obliterated by castration and restored by appropriate replacement therapy Hypothyroidism results in a moderately severe anemia in animals In the uncomplicated form the anemia is slightly macrocytic and associated with a hypoplastic bone marrow In clinical experience the anemia may be complicated by the secondary effects of achylia gastrica leading either to iron deficiency or to a deficiency in the anti pernicious anemia factor Hyperthyroidism causes some alterations in the white blood cells but has little effect on the red blood cell series Hyperactive states of the adrenal cortex may be associated with a mild polycythemia Adrenal steroids also have a marked lymphocytic effect causing the release of beta and gamma globulins from lymphoid tissue A mechanism involving the anterior pituitary and adrenal seems to exist controlling the release of antibodies under certain conditions It is suggested that other mechanisms also exist which control the number of circulating lymphocytes

Deficiency of the anterior pituitary secretions results in anemia in animals and man The anemia in animals is usually microcytic and hypochromic and may respond to several types of replacement therapy In man anemia develops slowly and is rarely severe Moderate reductions in the red blood cell count occur and the color index varies There is hypoplasia of the bone marrow The anemia in man does not respond uniformly well to the therapy now available but improvement often occurs with the replacement of end organ hormones

The preponderance of evidence indicates that the regulation of blood formation is not primarily under hormonal control The effects noted in various glandular disorders are due to alterations in metabolism produced in the bone marrow as well as all other body tissues

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EXTRAMEDULLARY BLOOD PRODUCTION

By Claus Munk Plum Ph D

IN MAMMALS throughout postnatal life erythrocytes and granulocytes are normally produced only in the bone marrow while the third type of circulating blood cell the lymphocyte is produced chiefly in the lymph nodes and the spleen and only to a relative small extent in the bone marrow During fetal life blood formation occurs to a large extent in organs other than the bone marrow It is well known that the liver and spleen take part in fetal blood formation and that this function ceases at birth but no one seems to have investigated the function of the mammary and prostate glands with regard to existence and duration of hematopoietic activity within them The observations to be reported at this time attempt to correlate the occurrence of extramedullary hematopoiesis in the mammary glands and the prostate with the hematopoietic functions of the liver and the spleen

Extramedullary blood formation by the newborn has been studied extensively in the past few years [1 10 11 1] Bertelsen[1] investigated the origin of the erythrocytes during the last fetal months and the first days of extrauterine life studying especially the liver spleen and the thymus gland Schlachta[17] found extramedullary foci in the prostate and the suprarenal glands Block in the kidneys and the renal pelvis Gruber[8] in the mammary glands and Weil [0] in the skin of the soles of the feet The observations by Marchand and Lohlein[14] of extramedullary foci in the greater omentum and the sole of the foot laid the foundation for all the more recent investigations of the problems of extramedullary blood formation These authors found that the perivascular cells perhaps similar to Saxer s primitive histiocyte [16] were the origin of the great stabformed types of basophilic and eosinophilic granulated cells and of the erythroblasts The occurrence of the erythroblast in the greater omentum however was denied by Seifert [18]

Weil s description of extramedullary hematopoiesis in the sole of the foot in human beings was based upon detailed investigations in only 4 cases [0] The cells of the hematopoietic foci were found about the sweat tubules or in the adipose tissue From his investigations the author concluded that the foci of blood formation always occurred in relation to the sudorific glands or to glands which might be a modification of sudorific glands e g mammary glands Weil s studies were continued by Dieterich [5] who especially observed the mammary glands and the skin of the hand foot head and axilla Dieterich s observations were based on a relatively large number of cases 4 fetuses aged _ to 6 months 14 newborn individuals 10 children aged 8 days to 3 years and 11 adults He found in 10 newborn infants that the pedal extramedullary foci were limited to the sole of the foot no foci being present in the dorsum of the foot This observation agreed with Weil s inference concerning the occurrence of hematopoietic tissue in relation to sudorific glands

From the Department of Pathological Anatomy University of Copenhagen Denmark

In the mammary glands Dieterich found hematopoietic foci which greatly resembled bone marrow These foci contained mainly cells of the myeloid and the lymphoid series erythroid elements being rarely found (in one 11 year old child however large numbers of erythroblasts were present) Such observations help to confirm the theory advanced by Morawitz and Rehn (quoted by Dieterich) concerning *a reciprocal action between the leukocytic and erythroid system* Extramedullary foci were rarely found in the skin of the hand and never in the skin of the back head or axilla (Dieterich[5]) Extramedullary foci were never found in the normal adults

As mentioned previously the formation of erythrocytes and granulocytes normally takes place in the bone marrow but this normal formation may be supplemented in pathologic cases by extramedullary blood formation Such ectopic hematopoiesis frequently takes place in the spleen and the lymph nodes less frequently in the liver and only rarely in the suprarenal glands kidney cartilages the broad ligaments and scattered throughout the adipose tissue of the organism In general the sites of extramedullary blood formation which are found normally in embryonic and fetal life are also the sites in which the phenomenon occurs under pathologic conditions in the infant and adult

Extramedullary hematopoiesis occurs under various pathologic conditions in adult mammals Recorded cases refer chiefly to man In infants and young children extramedullary hematopoiesis is often found in association with severe anemia [8] Various authors[4] [19] have reviewed the recent literature on ectopic blood formation in erythroblastosis fetalis In pernicious anemia during relapse extramedullary hematopoietic foci are regularly found in the spleen and liver [12] [15] In macrocytic anemias especially those associated with liver disease such foci are frequently found in the spleen [21] Extramedullary foci occur in osteosclerosis [6] in invasion of the bone marrow due to various causes [3] [10] [1] and in Hodgkin s disease [1] even when the anemia is not very severe Ectopic blood formation has been described in erythremia [21] hemolytic jaundice [9] and leukemia [1] Tumors of heterotopic bone marrow have been observed in adipose tissue of patients with anemia in severe sepsis [21] Extramedullary hematopoiesis has been produced experimentally by repeated bleeding and by chronic poisoning with blood destroying substances [3]

In general the extramedullary foci may be composed of erythroid elements myeloid elements megakaryocytes or of all three types of cells The ectopic hematopoiesis is often interpreted as a compensatory phenomenon evidence for such a theory being among other things the readiness with which such a change occurs in infants and young children in whom the bone marrow has little or no room for expansion

Present Investigations

The present report deals with the results of the examination of a total of 94 individuals including 79 fetuses from 6 months of age to birth and 15 children and adults from 1 day to 25 years of age All fetuses with erythroblastosis fetalis were omitted and only normal material was used The material was collected from the Department of Pathological Anatomy University of Copenhagen

EXTRAMEDULLARY BLOOD PRODUCTION

By CLAUS MUNK PLUM, PH D

IN MAMMALS, throughout postnatal life, erythrocytes and granulocytes are normally produced only in the bone marrow, while the third type of circulating blood cell the lymphocyte is produced chiefly in the lymph nodes and the spleen and only to a relative small extent in the bone marrow During fetal life, blood formation occurs to a large extent in organs other than the bone marrow It is well known that the liver and spleen take part in fetal blood formation and that this function ceases at birth but no one seems to have investigated the function of the mammary and prostate glands with regard to existence and duration of hematopoietic activity within them The observations to be reported at this time attempt to correlate the occurrence of extramedullary hematopoiesis in the mammary glands and the prostate with the hematopoietic functions of the liver and the spleen

Extramedullary blood formation by the newborn has been studied extensively in the past few years [1, 7, 10, 11, 1] Bertelsen[1] investigated the origin of the erythrocytes during the last fetal months and the first days of extrauterine life studying especially the liver spleen and the thymus gland Schlachta[17] found extramedullary foci in the prostate and the suprarenal glands Block in the kidneys and the renal pelvis Gruber[8] in the mammary glands and Weil [0] in the skin of the soles of the feet The observations by Marchand and Lohlein[14] of extramedullary foci in the greater omentum and the sole of the foot laid the foundation for all the more recent investigations of the problems of extramedullary blood formation These authors found that the perivascular cells perhaps similar to Saxer s primitive histiocyte [16] were the origin of the great stabformed types of basophilic and eosinophilic granulated cells and of the erythroblasts The occurrence of the erythroblast in the greater omentum however was denied by Seifert [18]

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From the Department of Pathological Anatomy University of Copenhagen Denmark

TABLE 1.—*Continued*

Age	Case no.	Sex	Weight	Length	Liver: Lob. foci (1)	Liver: Port. foci (2)	Spleen: Pulp Eryt. (3)	Spleen: Pulp My. foc. (4)	Spleen: Follicles Eryt. (5)	Spleen: Follicles My. foc. (6)	Breast	Prostate	Plantar pad	Suprarenal glands
			Gm	*cm*										
9 months	163	m	1400	43	37.7	1.1	14.2	0.8	0.2	0.1	0	+		
	191	m	1400	46	26.3	1.7	9.5	0.2	0.1	0.1	(+)	(+)	(+)	0
	244	m	1350	49	46.8	2.0	18.9	1.2	0.2	0.1	0	+		
	247	m	1550	43	18.2	1.0	9.2	0.1	0	0	+	+	+	+
	270	m	2200	45	11.4	0.4	7.3	0	0	0	0	(+)		
	273	m	1200	40	63.5	2.2	13.5	0.4	0.1	0.1	+	0	0	0
	274	m	1150	39	48.2	1.7	12.2	0.4	0.2	0.1	(+)	(+)		
	275	m	1600	47	8.0	0.2	4.3	0.2	0	0	0	(+)	+	(+)
	12	m	2300	46	18.2	0.5	16.2	0.2	0.2	0.1	0	+	(+)	0
	14	m	2400	48	61.4	1.8	27.2	0.3	0.2	0.1	(+)	+		
	32	m	2000	43	70.2	2.4	26.1	0.4	0.3	0.2	+	0	+	(+)
	39	m	2650	52	19.7	0.7	12.4	0.1	0	0	+	+		
Full grown	154	f	3500	54	5.8	0.2	9.2	0.1	0.1	0.1	+		0	(+)
	182	f	2500	52	11.4	1.2	6.9	0.2	0.4	0.1	++		+	
	179	f	2850	52	31.6	0.9	16.7	0.4	0.1	0	(+)		(+)	(+)
	189	f	3350	53	47.8	2.9	22.1	0.3	0.6	0.2	++		+	
	221	f	2400	50	8.2	0.6	7.9	0.1	0.2	0	(+)		(+)	(+)
	239	f	3400	54	6.6	0.8	7.2	0	0.2	0.2	0		0	0
	245	f	3100	51	35.8	1.4	16.3	0.1	0.1	0.1	0			
	208	f	2900	52	49.8	0.8	11.2	0	0.3	0.1	+			
	268	f	3100	50	18.2	1.2	9.2	0.3	0.1	0	++		+	+
	278	f	3200	51	9.4	0.4	7.2	0.2	0	0.1	(+)			
	287	f	2550	50	11.4	1.0	8.2	0	0.1	0	0			
	160	m	2500	48	7.6	0.6	2.3	0	0	0	0	(+)	(+)	(+)
	164	m	3200	50	10.1	1.2	4.3	0	0.1	0	0	+		
	168	m	3500	54	56.6	2.4	12.3	0.3	0.2	0.1	0	+	0	0
	178	m	3500	50	23.8	0.8	10.6	0.1	0.2	0.1	(+)	++		
	188	m	3350	50	16.2	0.8	3.4	0	0.1	0.1	(+)	(+)	(+)	0
	203	m	2800	50	13.7	0.8	5.9	0.1	0	0	(+)	+		
	246	m	3650	53	0.4	0.2	1.3	0	0	0	0	+	+	(+)
	248	m	3400	52	26.5	0.8	9.8	0.2	0	0	+	(+)		
	256	m	3600	53	11.5	0.9	11.3	0.1	0.2	0	0	(+)		(+)
	267	m	3700	55	33.2	1.3	14.5	0.1	0.1	0	(+)	0	0	
	276	m	3500	52	21.6	1.4	13.2	0.5	0.4	0.3	0	0		0
	285	m	3100	55	13.5	1.7	5.6	0.3	0.1	0.1	(+)	(+)		
	11	m	4500	57	1.6	0.2	1.2	0	0	0	0	+	0	0
	23	m	3300	54	3.5	0.4	2.3	0	0	0	0	+	(+)	0
1 day	177	f			1.4	0.2					+		0	0
1 day	283	f			0.6	0					(+)		(+)	0
1 day	295	f			1.2	0.2					+		0	0
26 days	319	f			0.8	0.1					(+)			
95 days	278	f			0.5	0.2					0			

TABLE I —*Extramedullary Hematopoietic Foci in Various Organs of 94 Individuals Including 79 fetuses and 15 Postfetal Subjects*

Age	Case no	Sex	Weight	Length	Liver: Lob foci (1)	Liver: Port foci (2)	Spleen: Pulp Eryt (3)	Spleen: Pulp Myeloc (4)	Spleen: Follicles Eryt (5)	Spleen: Follicles Myeloc (6)	B ea t	Pro-stat	Pl ta ped s	S pra e al gla ds
			Gm	cm										
6 months	24	f	750	30	138 2	1 9	33 9	0 3	0 2	0 1	++			
	46	m	570	27	203 5	2 4	49 6	0 4	0 2	0 1	(+)	++		
7 months	190	f	1000	39	80 3	1 2	23 2	0 4	0 3	0 2	++		+	
	218	f	950	36	102 5	2 3	36 0	0 4	0 3	0 1	++		+	+
	238	f	1100	39	59 6	1 9	29 5	0 3	0 2	0 2	+++		+	
	293	f	770	34	132 3	2 0	26 8	0 3	0 3	0 3	++		0	
	257	f	900	39	77 2	1 6	19 3	0 2	0 2	0 1	+++		0	+
	200	f	1050	40	62 5	2 1	27 7	0 3	0 2	0 1	++		+	
	201	f	950	39	100 0	0 9	15 2	0 2	0 3	0 1	+++		0	
	311	f	1300	36	26 2	2 7	12 9	0 2	0 2	0 1	+++		+	+
	243	f	970	34	90 9	0 6	33 2	0 3	0 3	0 1	++		(+)	+
	161	m	1100	42	92 7	0 9	26 2	0 3	0 2	0 1	+	++	+	+
	174	m	800	32	121 9	2 2	39 4	0 3	0 2	0 1	+++	+++	+	
	175	m	1100	38	79 5	1 3	22 3	0 2	0 1	0	+	++	(+)	+
	240	m	950	44	139 0	1 3	36 5	0 4	0 2	0 1	+	++	0	+
	241	m	800	43	152 0	1 0	40 7	0 4	0 4	0 2	+		0	+
	27	m	1800	39	31 0	0 9	13 6	0 2	0 1	0	(+)	+		
	47	m	1300	37	56 2	1 2	16 2	0 2	0 1	0	0	(+)		
8 months	153	f	1500	43	33 8	1 2	28 6	6 4	0 2	0	+		0	0
	157	f	2150	40	89 6	0 8	10 4	0 2	0 1	0 1	++			
	171	f	1100	38	77 4	0 6	19 6	0 3	0 2	0 1	+++		+	+
	173	f	1 00	42	35 7	0 7	14 2	0 2	0 1	0	++		+	(+)
	230	f	2100	45	29 4	0 4	21 4	0 1	0 3	0 2	++			
	288	f	2300	44	18 2	0 4	10 2	0 2	0 1	0	++		+	(+)
	312	f	2100	43	22 4	0 8	11 2	0 1	0 2	0 1	++			
	162	m	900	40	131 0	2 8	41 9	0 4	0 3	0 2	++	++	+	(+)
	167	m	2450	50	16 2	0 8	10 2	0 2	0 2	0	(+)	0		
	169	m	1350	41	90 2	1 3	17 2	0 3	0 2	0 1	+	+	(+)	(+)
	237	m	1400	40	71 8	1 3	23 7	0 3	0 2	0 1	(+)	++	+	(+)
	242	m	1750	43	43 2	1 2	33 4	0 4	0 1	0	+	++	(+)	(+)
	13	m	1900	46	21 4	1 0	11 1	0 1	0	0	+	++	+	(+)
	28	m	1650	42	80 3	1 7	33 1	0 2	0 1	0	(+)	(+)	0	
9 months	151	f	2700	5	14 7	0 8	7 8	0 7	0 1	0 1	+		(+)	0
	172	f	3200	50	2 4	0 6	2 9	0 1	0	0	+++		+	(+)
	183	f	1700	45	11 2	0 4	6 4	0 6	0 2	0 2	+		(+)	0
	204	f	1550	43	53 7	2 2	2 4	0 2	0 1	0 1	++			
	212	f	2000	46	16 2	0 9	5 6	0 2	0 3	0	+		(+)	(+)
	230	f	2150	45	5 8	0 6	3 8	0 6	0 1	0 1	(+)			
	239	f	3400	54	11 9	0 3	6 9	0	0 1	0	+			
	250	f	1450	43	33 2	2 6	10 2	0 5	0 1	0	++		(+)	(+)
	310	f	2250	46	6 3	0 8	6 7	0 5	0 1	0	++		(+)	(+)
	33	f	2800	52	22 2	0 8	13 7	0 2	0 1	0	0			

average of foci found per microscopic field using Leitz objective 3 ocular 8 was recorded in the tables In the liver hematopoiesis takes place partly within the

TABLE 2 —*Number of Hematopoietic Foci in the Liver of Fetuses of Various Ages (The numbers in parentheses are from Bertelsen [1])*

	Lobular foci							
Maximum	152 0	(160 0)	131 1	(144 3)	70 2	(92 5)	56 6	(49 8)
Minimum	26 2	(53 7)	16 2	(1 4)	2 4	(2 9)	0 4	(0 5)
Average	87 7	(97 5)	54 3	(66 2)	27 6	(37 2)	19 0	(24 2)
	Portal foci							
Maximum	2 7	(2 4)	2 8	(3 0)	2 6	(2 4)	2 9	(3 0)
Minimum	0 6	(0 6)	0 4	(0 7)	0 2	(0 2)	0 2	(0 5)
Averag	1 50	(1 48)	1 11	(1 36)	1 17	(1 18)	1 00	(1 34)
Age	7 months		8 months		9 months		Full grown	

TABLE 3 —*Number of Hematopoietic Foci in the Spleen of Fetuses of Various Ages (The numbers in parentheses are from Bertelsen)*

	Erythroblasts in pulp							
Maximum	40 7	(46 7)	41 9	(32 0)	27 2	(26 9)	22 1	(33 9)
Minimum	12 9	(9 1)	10 2	(6 2)	2 9	(4 2)	1 2	(2 5)
Average	26 2	(23 6)	20 4	(18 9)	11 5	(14 6)	8 8	(12 2)
	Erythroblasts in follicles							
Maximum	0 4	(0 8)	0 3	(0 7)	0 3	(0 4)	0 6	(0 4)
Minimum	0 1	(0)	0	(0)	0	(0)	0	(0)
Average	0 25	(0 26)	0 17	(0 18)	0 12	(0 13)	0 14	(0 16)
	Myelocytes in pulp							
Maximum	0 4	(1 2)	0 4	(0 9)	1 2	(0 6)	0 5	(0 8)
Minimum	0 2	(0)	0 1	(0)	0	(0)	0	(0)
Average	0 24	(0 36)	0 24	(0 27)	0 35	(0 25)	0 15	(0 23)
	Myelocytes in follicles							
Maximum	0 3	(0 5)	0 2	(0 4)	0 2	(0 3)	0 2	(0 5)
Minimum	0	(0)	0	(0)	0	(0)	0	(0)
Average	0 11	(0 09)	0 07	(0 08)	0 07	(0 09)	0 06	(0 08)
Ag	7 months		8 month		9 months		Full grown	

lobes (lobular) and partly in the periportal connective tissue (portal) so that it was necessary to make a differentiation between these two groups

The numbers of foci in the livers of fetuses of various ages have been retabulated in table 2 We give here only the maximum and the minimum and the average of the numbers given in table 1

TABLE 1 —*Continued*

Age	Case no	Sex	Weight	Length	Liver		Spleen				Breast	Prostate	Planta pedis	Suprarenal gland
					Lob foci	Port foci	Pulp		Follicles					
							Eryt	Myeloc	Eryt	Myeloc				
					(1)	(2)	(3)	(4)	(5)	(6)				
			Cm	*cm*										
1 day	437	m			0.9	0.2					0	(+)	(+)	0
11 days	339	m			2.1	0					0			
26 days	436	m			0.5	0					0	(+)	0	(+)
23 days	296	m			0.7	0.3					0			
42 days	291	m			0.3	0.2					0	(+)	(+)	0
5 months	393	m			0.2	0.1					0			
23 months	430	m			0	0.1					0			
6½ yr	396	m			0.1	0					0			
23 yr	206	m			0	0					0			
26 yr	203	m			0	0					0			

1) Average of 10 field of vision—lobular foci
2) Average of 10 field of vision—portal foci
3) Average of 10 field of vision—erythroblasts in the red pulp
4) Average of 10 field of vision—myelocytes in the red pulp
5) Erythroblasts/follicle
6) *Myelocytes/follicle*
+++ Hematopoietic foci in each of ten field of vision
++ Hematopoietic foci in more than 67% of the fields of vision
\+ Hematopoietic foci in more than 33% of the fields of vision
(+) Hematopoietic foci in less than 33% but more than 0% of ten field of vision
Blank space means no observation made in this case

The organs examined were as follows mammary glands liver spleen and in some cases the suprarenal glands and the soles of the feet After removal from the body the organs were fixed in 4 per cent formalin in 0 9 per cent sodium chloride for two days and then were treated as usual for embedding in paraffin For each organ 10–12 cuts were taken with intervals of 30 μ The 5–6 μ slides were stained with three different dyes as follows (1) hematoxylin eosin (2) van Gieson Hansen and (3) May Grunwald Giemsa The sections were examined under the microscope and a notation made of the presence or absence of foci of hematopoiesis in examination of a number of fields The particular magnifications used are given below

As a hematopoietic foci was counted all crowding of cells which belongs to the hematopoiesis the cells are usually in different stages of the development The number of cells varies

Results

The results of these investigations are listed in detail in table 1 Certain particulars are listed in tables 2 and 3

Liver It would have been of interest to obtain a measure of the amount of hematopoiesis in the liver but it was possible to obtain only a relative measure The

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The number of such foci shows a progressive decline with increase in age and during the last month of pregnancy the amount of hematopoiesis is markedly decreased, as seen in table 2

Spleen In the spleen the erythroblasts and the myelocytes were counted in the pulp and the follicles averaging the number found in 10 microscopic fields using Leitz objective 1/12 ocular 8 The results are tabulated in table 3 It will be seen that erythropoiesis in the spleen still takes place at the end of fetal life

Mammary glands In sections of the mammary glands twenty fields of vision were employed (Leitz objective 3 ocular 8) and the amount of hematopoiesis listed from zero (0) to 3 plus (+++) as explained in table 1

Although the material was small it allowed the conclusion that the hematopoiesis was maintained to greater extent in the female than in the male fetuses

These results agree with earlier investigations[5] [0] that the extramedullary foci occur in relation to the sudorific glands or their modifications e g the mammary glands

Prostate In the prostate extramedullary hematopoiesis was observed in anatomic relation to the lobules but the number of foci was not large The findings confirmed the observations of Schlachta[17] that it is normal to find extramedullary foci here The same seems to hold true for the mammary glands

The sole of the foot Only a small part of the material was used for these investigations Here there are small numbers of foci almost always in relation to the sudorific glands rarely in the adipose tissue

The suprarenal glands Only a few cases were examined In them, the extramedullary foci were very small and few in number

Comment

These observations on the rather persistent extramedullary hematopoiesis in the mammary and prostatic glands seems to indicate that the immature blood cells find similar conditions in the bone marrow and the stroma of the gland in question If this is so an explanation may be forthcoming why malignant epithelial tumors arising in the breast and the prostate metastasize to the bone (in particular to bone containing active blood forming marrow) In this connection it is of interest that extramedullary hematopoietic foci are present during fetal life in the lungs and the kidneys too and that bronchogenic carcinoma and hypernephroma also very often metastasize to bones

These statements are offered only as reflections Further investigations along these lines are in progress

Summary

Investigations of extramedullary foci of hematopoieses demonstrate that in human beings the extramedullary hematopoiesis in the fetus at term is more pronounced in the mammary glands of the female than in those of the male The author suggests the possibility that there may be a connection between the location of the foci and the liability of metastasis in cancer especially in mammary and prostatic cancer

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SOME HEMATOLOGIC EFFECTS OF IRRADIATION*

By William Bloom M D , and Leon O Jacobson, M D

DURING our study of the cellular changes in mammals exposed to external and internal sources of ionizing radiations evidence was sought for cor rectness of the idea that small doses of radiations stimulate cellular activity and multiplication Although this idea is held by a number of radiologists little basis for it is found in the radiologic literature other than misunderstanding and mis quotation of a few important papers Some of our experiments offered unusual opportunities for a consideration of the question of stimulation Thus, in those experiments in which there were progressive decreases in the amount of irradiation and in the reactions to focalized irradiation one could expect to find evidence of stimulating effects if they actually occurred But our findings in the organs and peripheral blood have been so consistently at variance with the idea of stimulation by small amounts of ionizing radiation that it seems desirable to summarize these observations

Some of the confusion on this question undoubtedly originates from a loose use of the word stimulation In the following discussion we shall discriminate between a primary stimulation which results directly in cellular hyperplasia hypertrophy or hyperactivity after irradiation without a stage of obvious previous injury and a secondary stimulation which might be considered as a reparative process resulting from the necrotizing action of radiation

Material and Methods

The radiations which were employed in these studies on animals and man fall essentially into two main categories (1) Externally originating total body ir radiation from x γ rays fast and slow neutrons (2) Focal irradiation by externally originating B rays and radioactive isotopes administered enterally or parenterally

1 *Total body irradiation* The organs of large numbers of rabbits rats and mice and those from a smaller number of guinea pigs were obtained for histologic study when the animals were sacrificed at intervals after varying dosages of x and γ rays fast and slow neutrons The general plan of these experiments was to start with the LD 50/30 days dose of each agent and to decrease it until no histologic or hema tologic changes were observed In most of the series the total amount of irradiation was given at one exposure although there were several large and important series in which small amounts of radiation were given repeatedly

We found it necessary to carry out these experiments for histologic purposes by killing the animals at planned intervals because cytologic examinations of animals

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Most of the work reported here was done in the histologi and hematologic groups of the Metal lurgical Laboratory of the University of Chicago and the Argonne National Laboratory Chicago under contract of the Manhattan District No W 31 09-eng 38 The detailed papers hich include references on which this communication is based ill be found in the *N t nal Nu l ar En gy S ies*

which were found dead were worthless The series was supplemented with the study of a number of moribund animals

2 *Focal irradiation* This occurred in the spleens and testes of a few mice given large amounts of irradiation by B rays from an external source of P^{32} However the great mass of our experiments with focalized irradiation effects occurred after the intravenous or intraperitoneal injection of radioactive isotopes In a few series these materials were given intramuscularly or by inhalation Among the elements studied were the α (Alpha) emitters Ra and Pu the β emitters P^{32} Sr^{89} Y^{91} and the β and γ (Gamma) emitters Zr^{95} Ba^{140} La^{140} Na^{24} and the degradation products of Ra These isotopes were given in dosages starting with the LD 50/30 days and in diminishing fractions thereof

The tissues were routinely fixed in Zenker formol imbedded in nitrocellulose and stained with hematoxylin-eosin azure II except for those portions of the tissues which were fixed in alcohol so that auto-radiographs could be prepared

Whenever possible studies of the peripheral blood were conducted on the same series of animals in which sacrifices were being made for cytologic study Where this was not possible groups of animals were studied in parallel series in still other experiments blood counts were made on the animal at the time of sacrifice in an attempt to correlate the peripheral hematologic picture with effects on the blood forming organs Sampling of peripheral blood was done at intervals varying with the chronicity of the experiment In all cases the technics employed were standard hematologic procedures

From the standpoint of the hematologic studies the experiments of most significance were (1) those in which an acute single dose of penetrating total body radiation was given (x rays or fast neutrons) and (2) the experiments in which rats guinea pigs mice and rabbits were exposed chronically to γ rays or x rays In one of the chronic gamma ray exposure experiments the radiation was given daily over an eight hour period and in another set of experiments the γ radiation was given twenty four hours per day In the chronic x ray experiments the daily exposure was given within a few minutes The daily exposure of these animals whether exposed to gamma rays or x rays extended over long periods up to four years

Observations

I CHANGES AFTER A WIDE RANGE OF DOSES OF TOTAL BODY IRRADIATION FROM EXTERNAL SOURCES

One of the effects of total body external irradiation in doses not higher than the LD 50/30 days is to separate those organs which are sensitive to this amount of irradiation and those which are not In the laboratory mammals which were studied with this dosage the radiosensitive organs are the gonads bone marrow spleen lymph nodes thymus parts of the gastrointestinal tract skin and bone Nerve muscle and most exocrine and endocrine glands are resistant [1]

It was found that all of the external ionizing radiations in equivalent doses produced similar effects [1] If the animals had been given the LD 50/30 days dose of x rays or γ rays or fast or slow neutrons the changes which resulted could not be

distinguished from one another That is in examining sections of the organs of these animals we could not tell which ionizing radiation had been used With this amount of irradiation the very first changes observed usually within the first hour were degenerative changes and death of cells in the blood forming organs bases of the intestinal crypts and foveollae of the stomach spermatogonia and ovarian follicular epithelium or developing ova and early mild inflammation (mainly edema) of skin

Mitoses were usually gone by the second hour There was no evidence of an increase in mitosis In rabbit bone marrow after 100r of total body x irradiation mitoses began to be present again after three hours They continued through the fourteen hour interval at a level slightly above that seen in controls At the eight hour stage some of the mitoses were abnormal During the next hours the debris increased in amount and was gradually phagocytized With an LD 50 dose after a few days the blood forming organs became markedly reduced in size and the bone marrow and lymph nodes became aplastic—often completely so

In the course of one to two weeks the blood forming organs gradually became actively hematopoietic again Only rarely did this process reach or exceed normal in the bone marrow Several months usually elapsed before the lymph nodes thymus and spleen returned completely to normal

With progressively smaller doses the degenerative effects were less marked They became minimal at 50r of x rays and were just detectible in our animals at 25r of x irradiation or the equivalent fraction of the LD 50/30 days dose of neutrons With doses lower than 25r no changes were found in animals so treated there was no evidence whatever for either an injurious or stimulating effect

In the regeneration after external irradiation the only instances of overcompensation of tissue was in bone marrow of rabbits which survived the LD 50/30 days In them the marrow seemed hyperplastic (both erythropoietic and myelopoietic within two months after exposure)

After the administration of single doses of either x rays or fast neutrons to rabbits mice and rats an increase in circulating heterophil leukocytes occurred within the first twenty four hours with doses up to and beyond LD 50/30 days In fact with doses of 500 and 800r in the rabbit Jacobson et al noted that two definite statistically significant peaks occurred at circa twelve and twenty four hours After doses of this magnitude a leukopenia invariably followed the heterophil leukocytosis With doses of 300r and below only a modest elevation in the number of circulating heterophils occurred and this at about twelve hours Control animals handled in a comparable manner (except for the actual exposure to irradiation) also had this latter modest peak increase after about twelve hours Heterophils are the only circulating cells which are initially increased in number after acute total body radiation Lymphocytes monocytes eosinophils reticulocytes and platelets are reduced

As recovery occurs in the hematopoietic organs however a compensatory increase above normal control values was not uncommonly encountered particularly for the heterophils and reticulocytes No significant absolute lymphocyte increase on a compensatory basis was encountered in experiments in which doses

of 800r to 5 r acute total body X radiation were administered In fact lymphocyte values in the peripheral blood of the rabbit are reduced over a period of ninety days after an acute exposure to 800r This is in agreement with the slow return of the lymph nodes to normal after irradiation

After acute doses of x rays or fast neutrons Jacobson et al [2, 3] observed an abortive rise in lymphocytes heterophils and reticulocytes in rabbits between the fourth and twelfth day after irradiation This rise may possibly represent an abnormal stimulation in the sense that certain precursors are sufficiently altered to produce a limited succession of abnormal progeny

Chronic Radiation Experiments

Chronic exposure of groups of rabbits mice and guinea pigs to γ rays in daily doses of 0 11 1 1 2 2 4 4 8 8 γ for eight hours per day or twenty four hours per day over periods extending beyond three years carried out by Lorenz et al [4] has not shown evid nce of a stimulating effect on the blood forming tissue which was reflected in the hematologic constituents of the periph ral blood Similar experiments were conducted with chronic daily exposure of rats to x radiation but different in that the daily exposures required only a few minutes [5] No evidence of stimulation was apparent in the peripheral blood of these animals In those instances in which an effect occurred it was invariably a reduction in the number of circulating cells

No deliberate or well controlled human experiments have been done which are comparable in chronicity to these animal experiments The experiments of Low Beer and Stone [6] and Nickson Cantril and Jacobson [7] however in which human subjects were exposed up to a total do e of 300r total body given in divided doses of from 5 to 20r (x ray) produced a general reduction in the various hematologic constituents of the peripheral blood In several cases studied by Low Beer and Stone [6] however an absolute monocytosis became apparent reaching in several instances more than 50 per cent of the circulating leukocytes It has not occurred in the human cases we have studied nor has it been seen in the many animal experiments referred to above

2 EFFECTS OF FOCALIZED IRRADIATION

In mice exposed to large amounts of β rays from plaques containing P there was some focalized damage in several organs adjacent to the skin Changes were very marked in ovaries and testes But the changes which are of interest in this communication occurred in the spleens of a few of the animals On the dorsal surface of the spleen there was a zone of severe radiation damage This zone gradually merged into normal spleen the gradation consisting of progressively diminishing damage without any evidence of a zone of hyperplasia of undamaged cells Since the β rays have only a limited range of penetration it would be expected that near the periphery of their range the small amount of radiation would evoke a stimulating effect if such an effect does occur

This lack of a stimulating effect at the periphery of an area of focalized radiation is also characteristic of the changes which occur after localized deposition of radioactive isotope When these isotopes accumulate in given areas in radiosen

distinguished from one another That is in examining sections of the organs of these animals we could not tell which ionizing radiation had been used With this amount of irradiation the very first changes observed usually within the first hour were degenerative changes and death of cells in the blood forming organs, bases of the intestinal crypts and foveollae of the stomach spermatogonia and ovarian follicular epithelium or developing ova and early mild inflammation (mainly edema) of skin

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Other statements are found in the literature which have affected the conception that x rays produce a stimulating effect on blood forming tissue These effects however have been described after large doses of penetrating x irradiation An increase in the erythrocytes per cu mm and hemoglobin in Gm per 100 ml [10] an increase in the platelets [11] [1] reticulocytes [12] [14] and polymorphonuclear neutrophils[15] have been described by a number of authors These and other papers on this subject however generally dealt with inadequate numbers of control and experimental animals and therefore deny statistical validation Reports are also found in the literature describing the findings in the peripheral blood of man after chronic exposure to ionizing radiation These reports stress the significance of a lymphocytosis [16] monocytosis [17] eosinophilia [18] [19] leukopenia [19] [20] [1] anemia either normocytic or macrocytic [22] [3] These reports have tended to instill a sense of security in those working with radiation because of the implication that peripheral blood findings could be used as an indication of effect on a given individual even with radiations in the tolerance range (0 1 r total body exposure per day) There is no work to substantiate this As significant as many of these studies have been the re-evaluation of these concepts is indicated at this time because of studies conducted on the Plutonium Project in the past few years

Our findings and interpretations are in accord with the general conclusions of Czepa [4] and Packard [5] As the latter points out The evidence now at hand points to the conclusion that radiations do not directly stimulate normal activities of the cells their primary effect is always an injury from which the cell may recover perfectly But the degeneration products may temporarily quicken the tempo of some normal processes such as protoplasmic streaming and mitosis an acceleration which is followed by a retardation and often by very obvious injury Such reaction is secondary and is not true stimulation in the sense in which the term is used in radiological literature

Conclusions

Extensive studies with acute and chronic application of externally originating ionizing radiations and internally deposited radioisotopes have failed to reveal evidence in the blood forming tissues and peripheral blood of a primary stimulation of hematopoiesis However secondary or compensatory increases in certain of the cellular constituents of the peripheral blood were seen and were invariably preceded by a reduction The initial leukocytosis (heterophil increase) which occurs in the first twenty four hours after acute exposure to externally originating irradiations is probably a reaction to injury mediated through a mobilization rather than a new formation of blood cells The abortive rise in heterophils lymphocytes and reticulocytes is likewise probably a result of frank injury

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sitive organs they produce localized areas of radiation damage—much more extensive with β emitters than with α emitters because of the longer range of the former The penetration of alpha rays is only a fraction of a millimeter in tissue whereas the β ray penetrates several millimeters In or near none of these areas of focalized damage is there any evidence of cellular stimulation as evidenced by hyperplasia on normal cells

With the exception of the highly diffusible Na [24] the majority of the isotopes studied tended to localize in bone and most of them also accumulated in the red pulp of the spleen and in other organs This resulted in a marked hypoplasia and aplasia of the bone marrow As a consequence most of the spleens of these animals showed a great increase in ectopic myelopoiesis over the normal This obviously is in compensation for the aplastic bone marrow In no instance was a primary stimulation produced in the blood forming tissue which reflected an increase in circulating cells Compensatory increases were however noted in a few instances The radioisotope Sr^{89} is fairly generally distributed to blood forming tissue within the first few days after its parenteral administration It produces a reduction almost immediately in the nucleated cells of the peripheral blood It translocates however to bone and therefore exerts its major effect on the bone and bone marrow The lymphocytes however, remain depressed for long periods even though normal lymphopoiesis is resumed in the lymph nodes and spleen This phenomenon occurs with other isotopes which are not localized more than temporarily in lymphatic tissue

In some of the rats to which Sr^{89} was administered osteogenic sarcomas de veloped eight to ten months later This might be considered as a stimulation

Discussion

It is clear from the histologic examination of our animals that there is no evi dence of a primary stimulation of hematopoiesis by small amounts of irradiation from either generalized external or focalized internal sources The changes in the cells of the circulating blood after irradiation point to the same conclusion although the analysis is complicated by factors of mobilization and localization in the various parts of the circulatory system The heterophil increase which fol lows rather large acute exposures to penetrating radiations is according to Isaacs [8] a hastened maturation of heterophil precursors and thus a stimulation

The mechanism of the spectacular monocytosis described by Low Beer and Stone[7] in certain of the humans exposed to divided doses of total body x irradiation is not clear Since it occurred only in individuals with degenerative arthritis it may be a response associated with the underlying disease process

Irradiation of the hematopoietic organs as reflected in changes in the peripheral blood is often stated in the literature to produce stimulating effects The articles most frequently referred to in this connection are those of Murphy and his co workers [9] Actually these authors stated We have further noted that by one small dose of x ray we could obtain in a certain proportion of animals a stimulation of the lymphoid elements preceded by a comparatively short period in which the lymphocytes were below normal

Other statements are found in the literature which have affected the conception that x rays produce a stimulating effect on blood forming tissue These effects however have been described after large doses of penetrating x irradiation An increase in the erythrocytes per cu mm and hemoglobin in Gm per 100 ml [10] an increase in the platelets [11, 1] reticulocytes [13, 14] and polymorphonuclear neutrophils[15] have been described by a number of authors These and other papers on this subject however generally dealt with inadequate numbers of control and experimental animals and therefore deny statistical validation Reports are also found in the literature describing the findings in the peripheral blood of man after chronic exposure to ionizing radiation These reports stress the significance of a lymphocytosis [16] monocytosis [17] eosinophilia [18, 19] leukopenia [19, 20, 21] anemia either normocytic or macrocytic [22] These reports have tended to instill a sense of security in those working with radiation because of the implication that peripheral blood findings could be used as an indication of effect on a given individual even with radiations in the tolerance range (0 1 r total body exposure per day) There is no work to substantiate this As significant as many of these studies have been the re-evaluation of these concepts is indicated at this time because of studies conducted on the Plutonium Project in the past few years

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Extensive studies with acute and chronic application of externally originating ionizing radiations and internally deposited radioisotopes have failed to reveal evidence in the blood forming tissues and peripheral blood of a primary stimulation of hematopoiesis However secondary or compensatory increases in certain of the cellular constituents of the peripheral blood were seen and were invariably preceded by a reduction The initial leukocytosis (heterophil increase) which occurs in the first twenty four hours after acute exposure to externally originating irradiations is probably a reaction to injury mediated through a mobilization rather than a new formation of blood cells The abortive rise in heterophils lymphocytes and reticulocytes is likewise probably a result of frank injury

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THE EFFECT OF RADIATION ON HEMOPOIESIS IS THERE AN INDIRECT EFFECT?[1]

By JOHN S LAWRENCE M D WILLIAM N VALENTINE M C A U S AND ANDREW H DOWDY M D

1 INTRODUCTION

SINCE the discovery of the x ray by Roentgen medical science has evinced great interest in elucidating its mode of action Today the problem is by no means completely solved Certain facts particularly those relating to the results obtain d when tissues are subjected to direct radiation are well established and widely accepted For example the aplasia of bone marrow resulting from direct wide spread radiation in large doses must be regarded as beyond dispute

However there are other observations both of a clinical and experimental nature which are poorly understood and highly controversial These relate particularly to changes in tissues far removed from the direct site of radiation and have l d to the postulation of an indirect action of x rays For example many roentgen ologists have privately observed and others have publicly reported marked involution of lymph nodes involved in a lymphomatou or metastatic process when radiation was given to oth r areas sufficiently remote as to have precluded any direct exposure of the former Equally well known is the marked reduction in the blood count of leukemias treated by x ray and the leukopenia which develops in frequently in similarly treated nonleukemic individuals even when supposedly very small areas of marrow are subjected to the effects of direct radiation These effects at least in superficial appearance are not unlike those well established for direct radiation The result has been a welter of interesting but confusing literature which will presently be reviewed

It is with this possibility of an indirect action of roentgen radiation that this report is concerned The concept of indirect action requires further definition It is inconceivable in an organism whose every cell is bathed in a continuous cir culating fluid medium and which is interlaced with an intricate network of nervous tissue that such an ag nt as x ray known to be capable of creating exten sive destruction of certain cells should be without an indirect effect It is well established that there are indirect effects from a thermal burn or mechanical crush Yet in the case of crush injuries at least the only action directly attribut able to the causative ag nt ceases at the time the trauma is discontinued What occurs in the organism after that must be regarded as strictly nonspecific even though these events may be of a highly serious nature at times We therefore

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[1] This paper is based on work performed under Contract No W 7401-eng 49 with the Manhattan Project for the Un ersity of Rochester Rochester New York It will appea in Division VI of the Manhattan Project Technical Series as a p rt of the contribution from the University of Ro h ster Pro ject Radiology Division

have limited our approach to an effort to demonstrate indirect effects *peculiar* to radiation The pertinent question is whether or not radiation results in the formation of specific substances (not merely those liberated by nonspecific cellular injury or death) which when transported to distant areas produce a characteristic indirect effect

If such distant indirect effects exist it seems reasonable that the agents mediating them will in all probability be transmitted to their site of action by way of the circulation The experimental approach has been therefore to arrange for the direct passage of a very large volume of blood for a period of several hours from a radiated to a normal animal and then subsequently to observe the normal animal for evidence of changes which might be interpreted as indirect radiation effects Cross circulation by way of carotid to carotid anastomoses satisfies the concept of this approach Parabiosis in which small circulatory connections undoubtedly exist between the two animals was felt to be disadvantageous in that only small amounts of blood cross circulate per unit of time The methods and technics employed will be described in detail below Important corollary principles are that the radiated animal must receive a relatively large acute dose of radiation in order that any indirect effect present may be made more manifest and that sufficient experiments must be done to cover all time intervals up to at least a few days after radiation of the one animal Significant changes in the leukocyte and lymphocyte picture in the normal animal following cross circulation have been selected as the criteria of an indirect effect in these investigations These criteria were selected because (1) these elements have been shown to be among the most sensitive indicators of damage by radiation and (2) changes in these elements and their precursors have been repeatedly mentioned by those attributing indirect effects to radiation

2 Review of the Literature

An appreciable amount of medical literature has accumulated concerning the indirect effects of x rays Particularly in the early 1900 s many contributions to the subject were making their appearance the German investigators being especially active in this field Much of this early literature is mutually contradictory and largely of historical interest Dosage of radiation administered was in most instances not accurately known and the quality of radiation employed varied widely from investigator to investigator Roentgenology was in its infancy and technical factors poorly understood and poorly controlled Nevertheless the field is not one that readily lends itself to experimental investigation and even with vastly increased knowledge and improved technical control the same general arguments prevailing soon after the turn of the century are still being debated in the literature of the last twenty years

Broadly these investigations can be grouped into four main types (1) The demonstration of or the failure to demonstrate specific toxins (most frequently leukotoxins) in the serum of patients or experimental animals exposed to radiation Both in vivo and in vitro studies of this nature are reported (2) The demonstration of or the failure to demonstrate significant histologic changes in tissues

ordinarily sensitive to roentgen radiation following radiation of some site remote from the tissues studied (3) The clinical demonstration of significant involution of susceptible tissues far removed from the point of application of direct radiation (4) A group of miscellaneous investigations chiefly concerned with demonstration of a wide variety of biochemical abnormalities occurring after radiation and purported to be the mediators of an indirect effect

Efforts to demonstrate a specific toxin developed as a result of radiation have been particularly numerous Linser and Helber [8] (quoted by Capps and Smith) found that a leukotoxin was produced in the blood of organisms exposed to x ray This leukotoxin when injected into other animals destroyed the circulating leukocytes and when added to animal exudates containing leukocytes caused loss of motion and degeneration of the cells Curschmann and Gaupp in 1905 injected into rabbits serum taken before and after radiation therapy from a patient with lymphatic leukemia A few hours after injection the authors observed leukopenia not present in control animals This they concluded was due to the development of a specific leukotoxin which could also be demonstrated as capable of destroying human leukocytes in vitro They presented evidence suggesting that such a leukotoxin was inactivated by heat (60 C for one half hour) The following year Klieneberger and Zoeppritz[20] were unable in their investigations to demonstrate any consistantly toxic action of radiated serum on leukocytes in vitro either as to cellular disintegration or as to influence on amoeboid activity In addition no constant leukopenic effect was observed after injection of radiated serum into rabbits The authors concluded no toxin could be demonstrated as a result of radiation Milchner and Wolff[29] observed some decrease in leukocytes in animals after the injection of 5 cc of serum from a radiated animal of the same species They also noted some degree of leukopenia when material from a radiated spleen was injected while injections of normal spleen resulted in leukocytosis However they felt that their results were not of sufficiently concrete nature to be strong evidence for the presence of a leukotoxin particularly in view of wide physiologic variability in the leukocyte counts of the experimental animals used Benjamin[4] and co-workers after radiating the ears of rabbits with large doses while shielding the remainder of the animal found leukocytosis and lymphopenia occurring but the blood picture returned to normal within twenty four hours They claimed to have found increased choline formation in animals after intense radiation and to have noted that this corresponded to the period of leukocytosis The investigations of Capps and Smith[8] were interpreted as favoring the concept of a leukotoxin These authors found leukopenia to result from the injection of a few cc of radiated serum from one animal into the abdominal wall of another This serum was also said to cause abnormal destruction of leukocytes in hanging drop preparations With this view Harris[1] concurred though presenting no experimental evidence of his own

In 1918 Dorn[10] reported that testicular and ovarian atrophy and leukopenia of considerable duration resulted from the injection of enzytol a borate of choline The author felt that the effects of radiation were exactly reproduced by this substance and that this offered strong support to the concept of choline as the toxic cause of radiation effects Walterhofer[51] likewise considered indirect effects of

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Osgood[44] in 194- conducted in vitro experiments designed to determine if indirect radiation effects on cells of bone marrow could be demonstrated He set up four human marrow cultures identical in every respect except one received no radiation in one both cells and medium (35 per cent human serum and 65 per cent balanced salt solution) were radiated in one only the medium was radiated and in one only the cells were radiated Quantitative hematologic studies indicated no indirect action of radiation on marrow cells suspended in the radiated medium

A number of histologic studies have also been made in which tissue remote from the direct site of radiation has been examined for evidence of indirect damage Nakahara and Murphy[41] found identical changes in both deep and superficial lymphoid tissue following small doses of soft x ays Since 96 8 per cent of the rays did not penetrate beyond a depth of 1 cm it was felt these changes in deep tissue could not be the result of direct action by the rays Jolly and Ferroux[19] in histologic studies made on lymphatic structures outside the fi ld of direct radiation were of the opinion that there was grave doubt that the slight changes noted were due to an indirect toxic action of radiation Rather it was their inclination to attribute such slight changes as might exist to diffused or secondary radiation Akaiwa and Takeshema[1] exposed popliteal lymph nodes of rabbits to various amounts of radiation leaving one side as a nonradiated control The changes in lymphoid tissue on both sides were noted at a variety of intervals after radiation The authors concluded that in the control nodes histologic changes identical with those in the radiated nodes occurred but that the reaction was much less intense and mu h slower on the nonradiated side In 1940 Hsu and Ma[18] radiated one f mur only of rats with doses varying from 1000r over a four day period to 5000r over a forty day period The day after the course of radiation was complete animals were sacrificed and the bone marrow of radiated and nonradiated femurs the submaxil lary lymph nodes and the spleen of each were examined In the nonradiated hemopoietic tissues changes of a hyperplastic nature interpreted as compensatory were noted Le Blond and Segal[2 4] found large doses of roentgen rays produced second ary changes in well shielded organs far distant from the point of impact These consisted of constant thymus and generalized lymphatic atrophy and adrenal hypertrophy and frequently of fatty infiltration of the liver and ulcerations of the stomach Such lesions were considered part of a gene al intoxication following radiation an l were felt to be similar to those described by Selye as occurring in the nonspecific alarm reaction developing after the application of a wide variety of injurious stimuli It is interesting (as has been noted with other noxious agents capable of eliciting an alarm reaction) that thymus and lymphatic atrophy was suppressed by adrenalectomy but the gastric lesions and general lethal effects of the rays increased Barnes and Furth[3] in 1943 using single and parabiotic mice reported an extensive series of investigations These authors examined bone mar row lymph nodes and spleen in unexposed areas of mice radiated with anywhere from 400r to 7000r Some histologic changes were ob erved but these were slight and regarded as probably the result of products of damaged tissue transported by way of the circulation The degree of change depended on the dose of radiation and probably on the volume of tissue radiated The authors observed similar histologic

radiation to result from some toxin, possibly choline In 1922 Billings[5] in discussing his experiences with the treatment of leukemia by roentgen ray stated that the application of radiation to any cutaneous surface resulted in the appearance of a leukolytic substance in the blood The serum of a treated patient dissolved white blood cells in vitro while the serum of an untreated patient had no such effect In the same year Murphy Liu and Sturm[31] were forced to conclude that their experiments failed to show any evidence of the presence of a lymphotoxin, even when exposures large enough to effect almost complete destruction of lymphoid tissue were employed Zacherl[56] four years later, as a result of experiments with radiation of single and parabiotic rats felt that some toxic substance capable of causing sickness and death of the animals must have been produced Szilard[49] in 1927 could demonstrate no autolytic effect on leukocytes in the serum of radiated leukemics nor could any such autolysin be demonstrated by complement fixation tests He could find no increase in choline in the blood or urine of leukemics under x ray therapy This author postulated none the less that perhaps electrons circulating in the blood exerted deleterious effects on distant sensitive cells Strumia s[47, 48] studies led him to conclude sweepingly in 1929 that radium emanations as such are carried by the blood or that they act through the production of leukotoxins which are produced by the direct effect of the radium emanations upon the white cells of the blood in superficial vessels or perhaps upon fixed cells of the reticulo-endothelial system He further stated The direct action of radiation upon hemopoietic foci is altogether unimportant In all cases the blood acts as a carrier of radiations either as such or modified to the hemopoietic foci where its main action takes place Woenckhaus[55] felt that leukopenia observed in normal rats after injection of serum from strongly radiated rats was due to products of proteolysis in the injected serum However he also found that when one member of a parabiotic pair was radiated and the other shielded a *leukocytosis* and not *leukopenia* occurred in the non radiated parabiont In 1932 Zwerg[58] heavily radiated skin flaps in small laboratory animals The remainder of the animal was shielded yet if the pedicles connecting the skin flap to the animals remained intact they uniformly died in two to nine days and exhibited varying degrees of leukopenia and lymphopenia The latter however were not of the order of magnitude uniformly seen in animals who have received enough generalized radiation to result in death Zwerg concluded that the effect of roentgen rays on the white blood cell picture is not a direct action on the blood forming organs and probably not on the circulating blood Rather he believed the decomposition of cells gives rise to toxins which in turn damage the hemopoietic system

Macht[7, 8] in more recent years has attacked the problem by phytopharmacologic methods examining blood and serum obtained both before and after radiation from a variety of animals His method briefly consists of studying the growth of roots of Lupinus albus seedlings in standard physiological solutions as compared with the growth of similar seedlings in similar solutions to which blood or serum had been added This investigator concluded that there is a toxic substance present in radiated but not in normal serum and that it reaches its maximum titer twenty four to forty eight hours after treatment disappearing in a few days

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changes in mice burned under anesthesia with the actual cautery over the chest and abdomen, and in the lymph nodes of two mice dying of iodoacetic acid poisoning In addition the effect on the hemopoietic tissue and lymph nodes of normal mice whose parabionts had been subjected to radiation was studied Histologic changes similar to but much less extensive than those found in the radiated animal were observed in the tissues of the nonradiated parabiont These were considered non specific Densted [9] likewise in 1943 reported on a large number of examinations of bone marrow and peripheral blood in patients being treated with x ray Control specimens were obtained before treatment The majority of the patients were suffering from malignant disease and x ray dosages while variable were for the most part cancericidal in magnitude Marrow specimens were obtained a considerable distance away from the sites exposed to direct radiation Twenty eight patients developed no granulocytopenia (below 3000 granulocytes per cubic millimeter) In these there was no abnormality of the nonradiated marrow either as regards total quantity of cells or differential values In 20 patients the granulocytes of the peripheral blood fell below 2300 per cubic millimeter In these cases the cellularity of the nonradiated marrow remained normal but adult polymorphonuclear leukocytes in the marrow decreased from an average of 16 per cent in control groups to 6 5 per cent in granulocytopenic groups The promyelocytes were questionably increased For these reasons the author concluded there is in certain patients some degree of maturation inhibition in myelopoiesis and that this is probably due to some toxic or anaphylactic factor He considers this to be an indirect effect of roentgen and radium rays on hematopoiesis and suggests it may occur more readily in patients who are in poor condition

While many roentgenologists have observed phenomena suggestive of an indirect radiation effect relatively few have seen fit to publish these clinical observations However Langer [3] has stated that he had observed indirect radiation effects on several occasions He cites the case of a boy treated with x ray for verruca simplex of both hands Although only one hand was treated the warts gradually disappeared from the untreated hand He refers also to a case reported by Baensch in 1922 where in a patient with primary carcinoma of the breast regional nodes disappeared even though shielded with lead rubber during radiation Scott[41] has reported to have observed frequently in cases where large areas were radiated the disappearance of lymphosarcomatous glands in regions receiving no radiation In one case he states this was manifest within half an hour after therapy

In addition to the above a miscellany of diverse mechanisms have been postulated as the mediators of indirect radiation effects In 1905 Musser and Edsall[32] concluded that the effect of x ray is not direct but is dependent on a reaction of the body subsequent to exposure They felt that the increased catalysis observed after radiation was a response in the form of an increased fermentative process and concluded that favorable results to x ray therapy could occur only when the body was capable of such response Demieville[8] concluded that an indirect effect of radiation probably conditioned through decomposition products was exhibited in the blood forming organs Petersen and Saelhof[3] in 1921 demonstrated increased serum titers of several enzymes after radiation and speculated whether some remote effects of

radiation may not be due to enzyme mobilization Hussey[17] found an uncompensated alkali excess in rabbits exposed to x rays and suggested alteration in acid base balance may be an important factor in their action Opitz[35] suggested that toxins produced by x rays may be agglutinated by tumor cells, thus impairing tumor growth Rahm and Koose[39] assumed chemical changes are initiated in the dead interstitial substances of the body and that these may be responsible for the end effects seen V Pannewitz[36] observed acidosis followed by slowly increasing alkalosis which persisted for several days after radiation Kluge and Zwerg [1] have suggested that mobilization of hormones particularly those of the hypophysis are important in producing the observed effects of radiation Selye[45] in an extensive review of general adaptation phenomena has pointed out that many of the metabolic changes described after radiation such as hyperglycemia decrease in blood cholesterol increase in ketone bodies in the blood elevation of the NPN and disturbances in acid base balance are seen with equal frequency as a response to many injurious stimuli such as traumatic shock exposure to cold burns drugs and solar rays This author feels such things represent nonspecific systemic phenomena elicited by injurious stimuli to which the organism is qualitatively or quantitatively not adapted Very recently Dougherty and White[13] have proposed that roentgen radiation exerts both a direct and indirect effect on lymphocytes and that the indirect action can be explained on the bases of the increased pituitary—adrenal cortical activity caused by radiation

It is readily apparent tha the literature weighs heavily on neither side of the question

3 Experimental Technic and Methods

The experimental animal employed was the cat and the chief experimental device cross circulation by way of carotid to carotid anastomoses In the twenty-six successful cross circulation experiments which form the basis of this report a normal animal was in each instance cross circulated with a radiated animal The latter received its radiation either during the actual time of cross circulation or at varying intervals prior to its establishment A control group in which normal cats were cross circulated had already been established in this laboratory in connection with experiments previously reported [24]

The technic of establishing cross circulation was as follows The animals were anesthetized with nembutal administered intraperitoneally The operative site was prepared and the carotid artery of each on one side isolated and dissected free A small rubber covered clamp was placed at the proximal and distal ends of the freed vessel and the art ry divided midway between the two clamps Both segments were then washed free of blood with isotonic citrat d saline solution In each animal the proximal arterial segment was cannulated by threading it through a Monel cannula everting the cut end of the vessel over the outside of the cannula and tying with fine 5-0 catgut The two animals were next brought together in such a way that the proximal arterial segment of each was directly adjacent to the distal segment of the other They were conveniently supported in this position by the use of small sandbags A bed for the anastomo es was readily formed by sutur

changes in mice burned under anesthesia with the actual cautery over the chest and abdomen and in the lymph nodes of two mice dying of iodoactetic acid poisoning In addition the effect on the hemopoietic tissue and lymph nodes of normal mice whose parabionts had been subjected to radiation was studied Histologic changes similar to but much less extensive than those found in the radiated animal were observed in the tissues of the nonradiated parabiont These were considered non specific Densted,[9] likewise in 1943 reported on a large number of examinations of bone marrow and peripheral blood in patients being treated with x ray Control specimens were obtained before treatment The majority of the patients were suffering from malignant disease and x ray dosages while variable were for the most part cancericidal in magnitude Marrow specimens were obtained a considerable distance away from the sites exposed to direct radiation Twenty eight patients developed no granulocytopenia (below 3000 granulocytes per cubic millimeter) In these there was no abnormality of the nonradiated marrow either as regards total quantity of cells or differential values In 20 patients the granulocytes of the peripheral blood fell below 2300 per cubic millimeter In these cases the cellularity of the nonradiated marrow remained normal but adult polymorphonuclear leukocytes in the marrow decreased from an average of 16 per cent in control groups to 6 5 per cent in granulocytopenic groups The promyelocytes were questionably increased For these reasons the author concluded there is in certain patients some degree of maturation inhibition in myelopoiesis and that this is probably due to some toxic or anaphylactic factor He considers this to be an indirect effect of roentgen and radium rays on hematopoiesis and suggests it may occur more readily in patients who are in poor condition

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This amount of radiation invariably results in the death of cats within four to seven days

In the majority of experiments one animal was radiated at some interval prior to cross circulation In seven experiments however cross circulation was first established and then one animal radiated while the other was shielded Shielding was accomplished by means of an appropriately shaped wooden frame covered with one fourth inch of lead plate An ionization chamber placed under the frame recorded the small amount of scatter radiation which escaped the shielding This varied in different experiments from a minimum of 20r to a maximum of 43r

It was customary to administer isotonic saline subcutaneously to both animals during cross circulation and at its conclusion In some instances penicillin in saline was also administered

4 Presentation of Data

In all twenty six successful cross circulation experiments were performed Of these all the normal animals except one were followed hematologically for a period of approximately twenty-eight days after return to their own circulation Cat number 153 had to be sacrificed about two weeks after cross circulation because of a wound infection With one exception each cross circulated team consisted of one radiated and one nonradiated member The experiment involving normal cat number thirty six who was cross connected in series with two radiated animals simultaneously constituted this exception In such a circuit blood from the normal animal passed to one radiated animal which in turn was connected to a second radiated animal, which likewise in turn supplied blood to the normal cat

The data pertinent to these experiments are especially suited for presentation in graphic and tabular form and this has been the mode of presentation selected

Figure 2 shows in graphic form the duration of cross circulation and the interval after radiation of one member of the team that cross circulation was established It can readily be seen that in the majority of experiments the duration of cross circulation was in the neighborhood of eight hours A few animals were cross circulated as long as ten hours one for as little as two hours and seven minutes The group of animals in which radiation of one partner took place at the time the cross circulation was functioning were connected for approximately three to five hours More experiments were performed during or shortly after the radiation of one member because it was felt that logically the most profound effects on the normal partner could be expected under these circumstances Nevertheless all the time intervals after radiation up to eighty two hours were covered

Table 1 presents detailed hematologic data from each experiment and indicates the total leukocyte and absolute lymphocyte count of each normal animal before and at varying intervals after cross circulation with a radiated partner The intervals selected were done so arbitrarily with an eye to conserving space without sacrificing a comprehensive and representative presentation of the data In addition in the experiments in which one animal was shielded and the other radiated during the period of cross circulation the amount of radiation recorded by the

ing together the strap muscles of the two animals Following this, the distal arterial segments were drawn over the outside of the cannulated proximal segments and tied about the cannula Clamps were then released and cross circulation allowed to function from two to ten hours The cross anastomoses thus established afforded a continuous endothelial pathway by which the blood from each cat supplied one side of the head of its partner and vice versa Figure 1 diagrams the method of anastomosis

The volume of blood traversing an anastomosis of this type, will, if uncompensated result in the exsanguination of an animal within a few minutes At the conclusion of the period of cross circulation the patency of each connection was always tested in situ by reapplying the clamps dividing the anastomoses *distal* to each cannula and releasing the clamps for a brief instant

Prior to an experiment control hematologic studies consisting of hemoglobin determinations and a total and differential white blood cell count were made *Only animals which were clinically well and which showed no gross hematologic abnormalities were used* During the period of cross circulation leukocyte counts

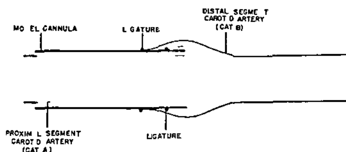


FIG 1 Diagram of end to end carotid to carotid anastomosis of the type employed in these experiments

and smears were made at approximately hourly intervals In each instance blood was obtained from the marginal ear vein The hematologic follow up of the non radiated animal consisted of a minimum of daily (except Sunday) total and differential white blood cell counts for a period of two weeks and similar studies made every other day for a period of approximately an additional two weeks All counts were made with Bureau of Standards certified pipets All blood smears were made by the coverslip method and stained with Wright s stain

The radiation to which one member of each cross circulated pair was subjected was kept constant and subject to the following factors

Total Dosage	*1500 r whole body radiation*
Voltage	k v p 250
Milliamperage	15
Target Distance	22 inches to center of cat
Filter	Aluminum parabolic plus ½ mm copper
Half Value Layer	2 1 mm copper
Rate of Administration	Approximately 25 r per minute with slight variation

TABLE 1.—T tal Leukocyt and Ab lute Lymphocyt C t Pe Cub M ll m ter f Twenty-s x
No mal C ts Follow g C s C culat n with Rat (3 4 mals

C t	Du t n f c c n hrs	Amt of R d tion	Pr r 1 a te	F rst After c	1 day	2 days	3 days	5 days	7 day	10-11 day	14 15 days	21 22 days	F d l Exp
109	3 0	35r	23 000 2 415†	64 000 2 560	41 000 1 230	35 200 3 23	19 100 1 034	23 100 1 848	79 600 3 623	21 900 2 080	29 900 2 691	27 300 3 003	13 500 1 670
1351?	6 0	20	14 700 5 408†		31 300 945	25 300 1 263		15 500 1 155	19 800 1 683	27 700 2 353	21 800 3 161	12 300 3 8 4	9 400 1 692
143	3 8	41	21 200 4 180†	18 600 84	22 600 2 260	21 700 1 696	15 000 900		22 000 2 470	14 8 1 1 6 8	13 800 3 558	17 700 2 301	34 00 3 470
146	3 7	29	16 470 4 410†	19 000 950	26 00 934	34 00 1 561	35 100 2 281		18 300 1 9 1	10 800 864	25 200 1 89	45 900 2 065	35 300 1 242
150	3 6	35	12 100 1 813†	55 000 2 750	17 100 171	16 200 213	11 000 9 5	26 000 80	19 300 1 447	23 000 805	12 800 344	8 200 1 129	25 400 386
153	3 3	21	5 600* 1 706†	19 430 3 783	21 500 1 592	15 400 2 464	25 30 3 606	3? 300 3 085	19 700 3 152	31 700 4 438			
1851?	5 7	24	7 800 1 560†	30 600 1 653	30 500 2 135	11 300 1 213	11 400 1 710		12 200 854	8 400 1 008	12 800 1 289	6 900 1 173	7 500 900
28	7 1	none	23 800 7 3 8†	54 500 5 450	68 800 8 256	59 500 7 735	30 750 9 562	34 300 6 174	29 600 5 032	44 900 6 286	21 700 4 774	14 300 6 435	12 000 4 200
33	5 0	no	15 200 4 408†	36 100 7 888	30 100 3 311	31 900 9 889	33 400 5 6 8	24 500 4 900	21 500 3 655	14 800 4 440	8 900 2 6 0	4 200 2 134	8 400 3 870
38	10 0		8 800 2 904†	28 900 1 445	79 000 2 610	8 300 849	21 100 2 541		15 900 3 498	1 400 2 108	7 800 3 3 4	6 000 4 080	15 100 4 551
64	9 5	n n	14 000 2 660†	28 300 849	13 800 1 932	24 500 3 6 5	16 200 3 240	16 500 2 640	11 000 2 310	13 00 4 521	28 500 7 980	12 700 4 318	14 600 5 256
63	10 0	e	20 800 3 824†	16 100 1 449	21 000 2 100	16 000 1 970	15 600 3 432	20 900 3 832	11 000 3 5 0	21 200 3 604	19 100 4 584	2 500 8 415	20 200 5 454
36	8 8	n	54 700 8 3 8†	14 000 1 870	22 500 3 150	3 300 2 499		19 500 5 870	33 900 3 107	35 000 3 500	26 100 3 654	15 600 4 524	19 900 3 587
73	8 0	non	13 200 3 641†	51 500 1 030	54 100 1 082	31 400 7 536	28 200 5 3 8	74 600 4 183	27 000 3 10	25 00 1 942	21 000 2 310	16 100 2 415	11 000 2 860
2	8 2	no	13 600 3 128†	50 800 2 032	34 300 3 097	30 900 4 635	27 400 4 998	39 200 3 920	2 300 5 352	2 100 5 525	16 700 5 010	10 600 1 908	22 700 2 270
32	8 0	n	15 00 3 551†	44 900 2 245	34 200 2 394	32 100 4 173	56 400 5 084		19 000 4 465	10 500 1 4 0	18 700 4 488	23 600 2 850	11 400 3 534
12	8 2	n n	13 800 5 512†		26 300 1 052		25 800 3 354	14 500 1 450	17 300 3 114	44 600 3 1	42 700 2 989	3 800 2 624	17 500 5 425
6	8 2		18 00 2 244†		30 00 2 149	45 000 1 20	26 00 2 136	19 000 1 10	16 600 3 652	25 600 2 560	20 100 2 010	14 300 2 860	13 200 1 188
31	8 5	non	13 300 3 26 †		20 600 2 884	31 700 1 9 3	15 200 3 168	14 360 1 16	10 600 2 1 0	19 000 5 130	10 300 2 266	10 300 2 266	10 200 2 856
30	8 0		16 300 2 608†		44 00 2 652	30 900 2 4		30 300 2 440	26 400 1 848	25 000 4 000	20 00 2 691	14 400 2 592	0 000 1 800
38	7 7		10 500 2 62 †		2 400 82		17 200 2 236	21 800 3 498		12 800 896	10 800 2 3 6	10 700 1 923	16 700 1 336

ionization chamber under the shield has been indicated In every experiment of this type a small amount of scatter radiation escaped the shielding

Tables 2 and 3 give the means and standard deviations for total lymphocyte and leukocyte counts respectively of the normal animals for the same time intervals before and after cross circulation as given for the individual animals in table 1 The figures are divided into three groups those applying to the shielded group of normals, which were cross circulated at the time of their partner s radiation, those applying to the non shielded group which were cross circulated with a previously radiated partner and those applying compositely to the group as a whole It will be noted that the number of animals for which data are presented varies somewhat on different days in the tables This is due to the fact that counts were made but six

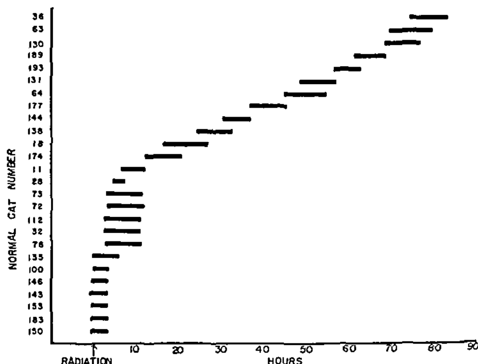


FIG 2 Each black line indicates the duration in hours of cross circulation in a single experiment The time interval after radiation of one member that cross circulation was established is also indicated

days a week so that at any specified interval after cross circulation it is possible that counts are not available for every member of the group

Figure 3 indicates graphically a composite picture of the average leukocyte and lymphocyte counts of the entire group during the period of follow up In addition the same data on the seven shielded animals are graphed separately both because these animals unavoidably received small amounts of radiation and because this type of experiment was considered the most critical of the group

5 RESULTS

Analysis of the data indicates few or no detectable hematologic abnormalities in the normal cat at any time after its cross circulation with a radiated animal

Table 1—T tal Leukocyt and Ab l t Lymphocyte C t P C 3 c M l m t of Twenty- x N rmal C ts F l wi g C t C lat with Rad 3 4 m s

C t N	Du ra tion of Ex po sure hrs	Amt of Ra- di- a- tion	Prior to Exposure	First After Exposure	1 day	2 days	3 days	5 days	[illegible] days	1[illegible] 11 days	14 15 days	21 days	End of Period
130	3 0	33r	23 000 2 415†	64 0[illegible] 2 560	41 000 1 [illegible]0	35 [illegible]00 1 32	19 100 1 054	23 100 1 848	[illegible] 000 3 6 5	21 9[illegible] 2 050	[illegible] 900 2 691	2 300 3 003	13 500 1 670
135	6 0	20r	14 [illegible]0 3 409†		31 500 945	25 3[illegible] 1 5		13 500 1 135	19 800 1 683	2 00 2 353	21 800 3 161	12 300 3 8 4	9 400 1 69
143	3 8	43r	21 [illegible]00 3 190†	19 600 83	22 600 2 60	21 [illegible]00 1 696	15 000 900		22 000 2 470	14 800 1 6 5	13 800 3 555	17 00 2 301	34 700 3 4 0
146	3 7	2 r	16 400 4 410†	19 000 950	6 3 934	34 00 1 561	35 100 2 291		18 300 1 9 1	10 800 864	25 200 1 897	15 900 2 065	3 500 1 242
150	3 6	35r	12 100 1 815†	55 000 2 750	17 100 171	16 200 243	15 000 9 5	26 000 80	19 300 1 447	23 000 80	12 800 344	8 200 1 179	25 400 356
153	3 3	23	5 600* 1 [illegible]9†	19 4[illegible]0 3 [illegible]3	24 500 1 592	15 400 2 464	25 [illegible]00 3 006	36 3[illegible]0 3 085	19 [illegible]00 3 152	31 00 4 435			
193	3 7	24r	7 800 1 560†	30 600 1 683	30 500 2 135	11 300 1 243	11 400 1 710		12 200 854	8 400 1 008	12 800 1 290	6 900 1 173	7 500 900
28	2 1	no e	23 800 7 378†	54 500 5 450	68 800 8 256	59 500 7 735	30 2[illegible]0 9 362	34 300 6 174	29 600 5 03	44 900 6 286	21 00 4 7 4	14 300 6 435	12 000 4 200
11	5 0	n e	15 200 4 409†	36 1[illegible] 2 888	30 100 3 311	31 900 9 889	33 400 5 678	24 500 4 900	21 500 3 655	14 800 4 440	8 900 2 6 0	4 200 2 184	8 400 3 870
15	10 0	non	8 800 2 904†	28 900 1 445	29 000 2 610	28 300 849	23 100 2 541		15 900 3 498	12 400 2 108	7 800 3 354	8 000 4 080	11 100 4 551
64	9 5	n n	14 000 2 660†	28 300 849	13 800 1 932	24 500 3 6 5	16 200 3 240	16 [illegible]00 2 640	21 000 2 310	13 00 4 5 1	8 500 7 990	12 700 4 318	14 600 5 256
63	10 0	n	20 800 5 824†	16 100 1 449	21 000 2 100	16 000 1 920	15 600 3 43	20 900 5 85	22 000 3 5 0	21 200 3 604	19 100 4 554	25 500 8 415	20 200 5 454
36	8 8	n	34 700 8 3 8†	14 000 1 870	22 500 3 150	3 00 2 499		29 100 5 8 0	23 900 3 107	35 000 3 500	26 100 3 654	15 600 4 524	19 900 2 587
73	8 0	e	13 200 3 564†	51 500 1 030	54 100 1 08	31 400 7 536	28 2[illegible]0 5 358	24 600 4 183	27 000 3 510	25 00 1 542	21 000 2 310	16 100 2 415	11 000 2 860
72	8 2	n	13 600 3 128†	50 800 2 032	34 300 3 097	30 900 4 635	29 400 4 998	39 [illegible]00 3 970	2 300 5 352	2 100 5 5 5	16 00 5 010	10 600 1 908	22 700 2 2 0
3	8 0		13 700 3 151†	44 900 2 245	34 200 2 394	32 100 4 173	16 400 5 084		19 000 4 465	10 500 1 4 0	18 700 4 488	15 000 2 850	11 400 3 534
12	8 2		13 800 3 312†		26 300 1 05		2 800 3 354	14 500 1 450	17 300 3 114	44 600 3 122	4 700 2 989	3 800 2 6 4	17 500 5 425
76	8 2	o	18 700 2 244†		30 700 2 149	43 000 1 770	26 700 2 136	19 000 1 710	16 600 3 6	2 600 2 560	20 100 2 010	14 300 2 860	13 700 1 188
31	8 1		12 100 3 267†		20 600 2 884	21 700 1 9 3	13 200 3 168	14 300 1 716	10 600 2 1 0	19 000 5 130	10 300 2 266	10 300 2 266	10 200 2 856
30	8 0		16 300 2 608†		44 00 2 682	30 900 2 472		30 500 2 440	26 400 1 848	25 000 4 000	20 700 2 691	14 400 2 592	20 000 1 800
38	7 7		10 500 2 625†		27 400 82		17 200 2 236	21 800 3 498		12 800 896	10 800 2 3 6	10 700 1 9 8	16 700 1 336

TABLE 1—Cont n ed

Cat No	Dura t on of x circ in hrs	Amt of Ra dia tion	Prior to x ci c	First After x circ	1 d y	2 days	3 days	5 days	7 days	10 11 days	14–15 days	21 22 days	End of Exp
44	6 3	none	11 900		14 300		13 100	23 600	20 300	11 400	9 300	13 ,00	20 500
			3 332†		1 287		2 358	2 124	3 248	2 052	1 023	959	3 485
4	8 0	none	10 900	27 000	29 000		13 400	8 500	11 300	10 100	6 300	5 00	12 600
			1 090†	0	0		1 742	1 10	1 582	1 111	630	798	1 764
177	8 2	none	5 900		11 200		14 700	18 200	11 00	15 100	17 200	15 500	15 200
			2 478†		1 680		2 35	2 730	02	2 265	3 268	3 255	3 648
189	7 0	none	6 000	22 800	17 800	14 500	14 500	23 600	15 600	18 900	17 300	24 700	15 300
			2 040†	0	1 780	1 740	2 030	1 888	624	1 512	2 768	1 9 6	1 989
193	5 8	none	10 200		15 500	25 900	12 000	15 000	14 800	10 100	13 800	17 00	12 400
			3 162†		3 410	7 770	3 120	2 2 0	2 2 0	2 626	4 830	4 248	3 770

= Total Leukocyte count
† = Absolute Lymphocyt c u t
‡ = Shielded mals

TABLE 2 —*Means and Standard Deviations for Absolute Lymphocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals*

	Sh lded Group			Non sh lded Gro p			Ent e Group		
	N	Mean	S D	N	Mean	S D	N	Mean	S D
Prior to x circ	7	2642 3	1063 1	19	3552 8	1798 2	26	3307 7	1664 0
First after x circ	6	2093 8	1145 2	11	1746 2	1515 0	17	1868 9	1368 9
1 day after x circ	7	1323 9	734 8	19	2403 6	1697 3	26	2112 9	1562 8
2 days after x circ	7	1386 3	665 1	14	4183 3	2892 4	21	3251 0	2719 5
3 days after x circ	6	1804 3	1159 5	17	3658 2	1928 2	23	3174 6	1924
5 days after x circ	4	1712 0	1017 4	17	3199 4	1657 7	21	1916 1	1646 8
7 days after x circ	7	2157 4	974 4	18	2975 5	1320 4	25	2746 4	1270 0
10–11 days after x circ	7	1882 3	1279 2	19	3066 8	1579 1	26	2747 9	1573 3
14–15 days after x circ	6	2160 2	1221 8	19	3351 3	1675 1	25	3065 4	1638 6
21–22 days after x circ	6	2257 5	1063 7	19	3191 3	1849 6	25	2967 2	1722 5
End of Experiment	6	1551 7	1056 8	19	3252 3	1346 4	25	2844 1	1463 5

N = Number of animals on which counts are available at the time specified in column 1

The average leukocyte count for the entire normal group and for the critical group in which cross circulation was established at the time of radiation of their partner remains throughout the entire approximately twenty-eight day period of follow up actually somewhat higher than the pre cross circulation control level In not even a single animal did evidence of significant leukopenia develop at any time during the experiment — this despite the fact animals received for a period of several hours the full volume output of the carotid artery from a radiated partner As would be expected well marked leukocytosis was present in many instances for a few days after the operative procedure Its duration varied rather widely from

TABLE 3 —*Means and Standard Deviations for Total Leukocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals*

	Shielded Group			Non-sh elded Gro p			Entire Gro p		
	N	Mean	S D	N	Mean	S D	N	M n	S D
Prior to x circ	7	14318 6	6461 4	19	14426 3	6658 5	26	14400 0	64 6 4
First after x-circ	6	34433 3	20133 3	11	34081 8	14440 3	17	34205 9	16032 1
1 day after x-circ	7	27700 0	7624 3	19	28,00 0	14484 4	26	28430 8	12833 4
2 days after x circ	7	22 57 1	9440 5	14	30450 0	11116 7	21	27885 7	11060 1
3 days after x-circ	6	20133 3	8 14 5	17	20185 3	,254 9	23	20571 7	7452 4
5 days after x-circ	4	14725 0	9385 2	17	22241 2	7860 7	21	22714 3	7977 7
7 days after x circ	7	20042 9	4988 9	18	19266	5510 8	25	19484 0	5278 3
10–11 days after x circ	7	19757 1	8704 6	19	20678 9	10798 4	26	20430 8	10115 0
14 15 days after x circ	6	19383 3	7313 0	19	1 736 8	8665 8	25	18132 0	8246 8
21 22 days after x-circ	6	19716	14826 0	19	14831 6	68 2 4	25	16004 0	9160 2
End of Experiment	6	21000 0	12572 7	19	14994 7	4553 6	25	16436 0	7261 5

N = Number of animals on which counts are a a lable at the time specified in column 1

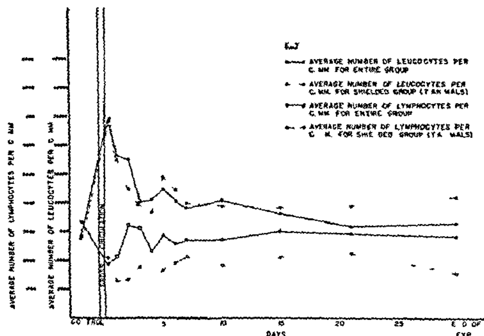


FIG 3 A erage leukocyte and lymphocyte counts per cubic millimeter of the whole group of animals for the entire period of the experiment are ind cated The a erage leukocyte and lymphocyte counts for the critical shielded group are graphed separately

animal to animal Of some interest is the very striking degree of leukocytosis developing at the time of cross circulation in certain animals whose partners had received their radiation at the time of or shortly before cross circulation Whether these more striking instances of leukocytosis are due to the passage of leukocytotic

TABLE 1—Continued

Cat No	Duration of x circ in hrs	Amt of R lation	Prior to x circ	First After x circ	1 day	2 days	3 days	5 days	7 days	10 11 days	14-15 days	21 22 days	End of Exp
44	6 3	none	11 900 3 332†		14 300 1 297		13 100 2 355	23 600 2 126	20 300 3 215	11 400 2 052	9 300 1 0 3	13 00 959	20 300 3 485
74	8 0	none	10 900 1 090†	27 000 0	29 000 0		13 400 1 74	8 500 1 105	11 300 1 592	10 100 1 111	6 300 630	5 00 795	12 600 1 64
177	8 2	none	5 90 2 4 8†		11 200 1 680		14 00 2 352	18 200 2 30	11 00 0	15 100 2 265	17 200 3 265	15 500 3 255	15 200 3 645
189	7 0	none	6 000 2 040†	22 800 0	17 80 1 50	14 500 1 740	14 500 2 030	23 600 1 855	15 600 6 4	15 900 1 512	17 300 2 64	21 700 1 9 6	15 300 1 997
193	5 8	none	10 200 3 16 †		15 500 3 410	25 900 7 7 0	12 000 3 170	15 000 2 250	14 800 2 270	10 100 2 6.6	11 800 4 810	17 700 4 215	12 400 3 20

* = Total Leukocyte count
† = Absolute Lymphocyte count
‡ = Shielded animal

TABLE 2 — *Means and Standard Deviations for Absolute Lymphocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals*

	Shielded Group			Non-shielded Group			Entire Group		
	N	Mean	S D	N	Mean	S D	N	Mean	S D
Prior to x circ	7	2642 3	1063 1	19	3552 8	1798 2	26	3307 7	1664 0
First after x circ	6	2093 8	1145 2	11	1746 2	1515 0	17	1868 9	1368 9
1 day after x circ	7	1323 9	734 8	19	2403 6	169 3	26	2112 9	1562 8
2 days after x circ	7	1386 3	665 1	14	4183 3	2892 4	21	3251 0	2 19 5
3 days after x circ	6	1804 3	1159 5	17	3658 2	1928 2	23	3174 6	1924
5 days after x circ	4	1712 0	1017 4	17	3199 4	1657 7	21	2916 1	1646 8
7 days after x circ	7	2157 4	974 4	18	2975 5	1320 4	25	2746 4	12,0 0
10–11 days after x circ	7	1882 3	12 9 2	19	3066 8	1579 1	26	2747 9	1573 3
14–15 days after x circ	6	2160 2	1221 8	19	3351 3	1675 1	25	3065 4	1638 6
21–22 days after x circ	6	2257 5	1063 7	19	3191 3	1849 6	25	2967 2	1722 5
End of Experiment	6	1551	1056 8	19	3252 3	1346 4	25	2844 1	1463 5

N = Number of animals on which counts are available at the time specified in column 1

The average leukocyte count for the entire normal group and for the critical group in which cross circulation was established at the time of radiation of their partner remains throughout the entire approximately twenty-eight day period of follow up actually somewhat higher than the pre cross circulation control level In not even a single animal did evidence of significant leukopenia develop at any time during the experiment — this despite the fact animals received for a period of several hours the full volume output of the carotid artery from a radiated partner As would be expected well marked leukocytosis was present in many instances for a few days after the operative procedure Its duration varied rather widely from

Table 3 —*Means and Standard Deviations for Total Leukocyte Counts of Normal Cats Following Cross Circulation with Radiated Animals*

	Shielded Group			Nonshielded Group			Entire Group		
	N	Mean	S D	N	Mean	S D	N	Mean	S D
Prior to x circ	7	14328 6	6461 4	19	14416 3	6658 5	26	14400 0	64,6 4
First after x circ	6	34433 3	20133 3	11	34081 8	14440 3	17	34205 9	16032 2
1 day after x circ	7	27700 0	7624 3	19	28700 0	14484 4	26	28430 8	12853 4
2 days after x-circ	7	22737 1	9440 5	14	30450 0	11216	21	27885 7	11060 1
3 days after x circ	6	20133 3	8714 5	17	20185 3	7254 9	23	20171 7	7452 4
5 days after x circ	4	24725 0	9385 2	17	22241 2	7960 7	21	22714 3	7977 7
7 days after x circ	7	20042 9	4988 9	18	19266 7	3510 8	25	19484 0	5278 3
10–11 days after x circ	7	19 57 1	8 04 6	19	20678 9	10798 4	26	20430 8	10115 0
14 15 days after x circ	6	19385 3	7323 0	19	17 36 8	8665 8	25	18132 0	8246 8
21 22 days after x-circ	6	19716	14826 0	19	14831 6	6872 4	25	16004 0	9 60 2
End of Experiment	6	21000 0	125 2 7	19	14994 7	4153 6	25	16436 0	7261 1

N = Number of animals on which counts are available at the time specified in column 1

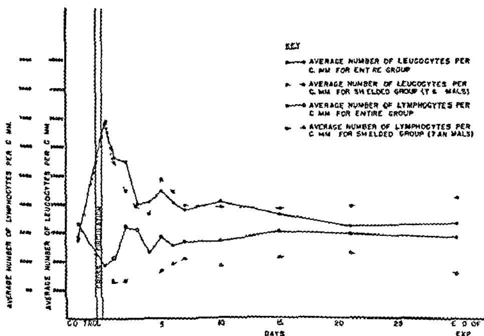


Fig 3 Average leukocyte and lymphocyte counts per cubic millimeter of the whole group of animals for the entire period of the experiment are indicated The average leukocyte and lymphocyte counts for the critical shielded group are graphed separately

animal to animal Of some interest is the very striking degree of leukocytosis developing at the time of cross circulation in certain animals whose partners had received their radiation at the time of or shortly before cross circulation Whether these more striking instances of leukocytosis are due to the passage of leukocytotic

metabolites resulting from tissue breakdown as a result of radiation cannot be said with certainty The leukocyte counts in question were markedly in excess of those developed in a normal control group however

Individual lymphocyte values found in normal animals after cross circulation show a moderate amount of variation As would be expected during the period of marked leukocytosis during the few days immediately following cross circulation a drop in the absolute lymphocyte count was noted Lymphopenia is a well known companion of neutrophilic leukocytosis and in this instance it was presumably accentuated by the fact some animals had been cross circulated with partners who had no circulating lymphocytes at all To be sure the average lymphocyte level for the entire group remained throughout slightly lower than that found before cross circulation and this trend was slightly more apparent in the critical shielded groups as a whole In view of the physiologic variations in lymphocyte counts in view of the persistence of some *increase* in the average total leukocyte counts throughout the experiment and more particularly in view of the fact that the reduction in lymphocyte count was relatively slight and not at all of the order of magnitude associated with direct radiation there is grave question that any significance can be attached at all to this finding At no time did the average per cent of lymphocytes fall below 4 9 per cent of the leukocytes present even in the shielded group and this figure was obtained only for the first day after radiation In all other instances it was appreciably higher than this

6 Discussion

It is apparent that under the conditions of our experiments no specific in direct effect of radiation has been demonstrated It is also felt that if significant indirect effects peculiar to radiation are dependent upon the circulation they should have been readily demonstrated by the experimental setup employed However it cannot be denied that had it been possible to maintain cross circulation for much longer periods —say forty-eight or seventy two hours instead of eight to ten hours—indirect effects too slight for demonstration during the shorter period might have become manifest The possibility of an indirect radiation effect mediated solely by the lymphatics has also been considered but discarded as highly unlikely Any lymphatic disseminated agent would in all probability soon reach the venous and arterial tree and would be expected to manifest itself in the blood Further if in some devious manner it should be removed from lymph before reaching the blood indirect effects of radiation would exist purely in the regional lymphatic drainage area of that portion of the body directly radiated The existence of such a purely localized regional indire t radiation effect is supported by no evidence and in fact is contrary to whatever evidence there is suggestive that such indirect effects exists

It is also apparent that there is no satisfactory evidence for a *characteristic* indirect radiation effect presented in the medical literature Such evidence as is presented is of dubious character and refuted for the most part by contrary results obtained in similar types of experiments by other investigators Nor has there been more than a desultory attempt on the part of investigators of indirect radiation effects to

differentiate body reactions common to a variety of damaging stimuli from those specific to radiation. This is a fundamental difference too, particularly in view of Selye's now well supported observations that an identical nonspecific systemic reaction, at times severe enough to result in death, occurs as a response to many unrelated noxious stimuli. Still, it is undoubted that leukopenia develops in a certain number of individuals treated with roentgen radiation even when the amount of marrow directly exposed to radiation is thought to be minimal. It is also highly likely that involution of and histologic changes in normal and abnormal lymphoid tissue remote from the site of direct radiation as observed by many roentgenologists and research workers is real and not apparent. It would seem wise to examine these considerations more closely to see if it is necessary to assume they are peculiar to radiation and if they can be accurately described as specific attributes of radiation per se.

Kornblum[illegible] and his associates in an extensive series of clinical observations concluded that therapeutic radiation tended to lower the leukocyte count. However, in approximately one-half of the patients studied there was no decrease in the number of neutrophils and in the great majority of the remaining cases depression was of slight degree. In but [illegible] cases out of [illegible] were the neutrophils reduced below 2000 per cubic millimeter. The effect of therapeutic radiation on the lymphocytes was more striking, a definite decrease in their numbers being the rule. In most cases the drop in count was relatively slight but in some instances it was very pronounced. It is thus seen that leukopenia of appreciable degree is an inconstant feature of radiation therapy applied to areas where there are no or minimal amounts of hematopoietic tissue. While the area treated, the volume of tissue radiated and the total amount of radiation are in our opinion all of importance as regards the production of leukopenia, yet it is impossible to predict accurately in most instances of local radiation over essentially non-hematopoietic areas which individuals will and which will not develop leukopenia. On the other hand, one can always anticipate the development of leukopenia when radiation is given over any appreciable amount of hematopoietic tissue such as in total body irradiation. Further, leukopenia associated with total body irradiation is more marked than when no or minimal amounts of hematopoietic tissue are radiated. Thus radiation over large amounts of active bone marrow can be expected to produce marked leukopenia, whereas radiation over non-blood forming areas produces mild, if any, leukopenia. Still another difference between the results of radiation over hematopoietic and nonhematopoietic areas is to be found in the morphological appearance of the bone marrow. With radiation directly over the bone marrow hypocellularity and/or aplasia result, whereas the marrow shows normal cellularity or it may even be hyperplastic when other tissues are exposed and it is excluded.

Three possible explanations for these differences arise. First, they may be only quantitative, the so-called indirect effect actually being a direct effect resulting from inclusion of larger amounts of hematopoietic tissue in the field of radiation than generally considered to be the case. It should be noted in this connection that the exact amount of hematopoietic tissue exposed to radiation is not known in

metabolites resulting from tissue breakdown as a result of radiation cannot be said with certainty The leukocyte counts in question were markedly in excess of those developed in a normal control group however

Individual lymphocyte values found in normal animals after cross circulation show a moderate amount of variation As would be expected during the period of marked leukocytosis during the few days immediately following cross circulation a drop in the absolute lymphocyte count was noted Lymphopenia is a well known companion of neutrophilic leukocytosis and in this instance it was presumably accentuated by the fact some animals had been cross circulated with partners who had no circulating lymphocytes at all To be sure the average lymphocyte level for the entire group remained throughout slightly lower than that found before cross circulation and this trend was slightly more apparent in the critical shielded groups as a whole In view of the physiologic variations in lymphocyte counts in view of the persistence of some *increase* in the average total leukocyte counts throughout the experiment and more particularly in view of the fact that the reduction in lymphocyte count was relatively slight and not at all of the order of magnitude associated with direct radiation there is grave question that any significance can be attached at all to this finding At no time did the average per cent of lymphocytes fall below 4 9 per cent of the leukocytes present even in the shielded group and this figure was obtained only for the first day after radiation In all other instances it was appreciably higher than this

6 Discussion

It is apparent that under the conditions of our experiments no specific in direct effect of radiation has been demonstrated It is also felt that if significant indirect effects peculiar to radiation are dependent upon the circulation they should have been readily demonstrated by the experimental setup employed However it cannot be denied that had it been possible to maintain cross circulation for much longer periods—say forty-eight or seventy two hours instead of eight to ten hours—indirect effects too slight for demonstration during the shorter period might have become manifest The possibility of an indirect radiation effect mediated solely by the lymphatics has also been considered but discarded as highly unlikely Any lymphatic disseminated agent would in all probability soon reach the venous and arterial tree and would be expected to manifest itself in the blood Further if in some devious manner it should be removed from lymph before reaching the blood indirect effects of radiation would exist purely in the regional lymphatic drainage area of that portion of the body directly radiated The existence of such a purely localized regional indirect radiation effect is supported by no evidence and in fact is contrary to whatever evidence there is suggestive that such indirect effects exists

It is also apparent that there is no satisfactory evidence for a *characteristic* indirect radiation effect presented in the medical literature Such evidence as is presented is of dubious character and refuted for the most part by contrary results obtained in similar types of experiments by other investigators Nor has there been more than a desultory attempt on the part of investigators of indirect radiation effects to

of recent investigations suggesting that lymphoid structures and possibly the lymphocytes may in some way be affected by the administration of adrenal cortical hormone [15, 11, 1, 44, 52, 51, 43, 14] In view of the above observations it is possible that x ray is merely one of many injurious agents capable of producing generalized involution of lymphoid tissue as a nonspecific response to local injury This of course is not to be confused with local involution and degeneration of lymphoid tissue directly exposed to radiation

The question of whether or not a specific indirect effect of radiation exists must be regarded as unsettled It is our feeling that leukopenia when it results from radiation is probably due at least in part to direct exposure of some of the hematopoietic tissue Other factors which may be operative are the general physical status of the patient nutritional disturbances resulting from the effects of radiation to the gastrointestinal tract general radiation sickness and the amount and degree of total body irradiation resulting from scattered radiation We also feel that morphologic changes occur in certain nonexposed tissues (e g lymph nodes and thymus) We are not convinced however that these nonspecific changes produce leukopenia

7 Summary

1 The general aim of the investigations here reported has been to obtain evidence for or against indirect radiation effects

2 To this end twenty-six successful cross circulation experiments (carotid to carotid anastomoses) have been performed between normal cats and radiated cats

3 Cross circulation was established in most instances at some specified time interval after the radiation of one partner All intervals up to eighty two hours after radiation of one partner were covered

4 In seven experiments cross circulation was established and then one animal radiated while the other was shielded These were considered the most critical experiments of the group

5 Detailed data on leukocyte and lymphocyte counts in the normal animals obtained during an approximately twenty-eight day period of follow up are presented

6 These data are not considered to support the thesis of indirect effects peculiar *to radiation A trend toward slightly lowered absolute lymphocyte* counts in normal animals after cross circulation was not considered significant and in no instance did leukopenia develop in the normal animal

7 The literature is reviewed and discussed

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many instances where an indirect effect is said to occur It may well be that the amount of blood producing tissue radiated in these cases is sufficient to reduce the output of blood cells to a point where their normal level cannot be maintained In particular one must consider the possibility that in debilitated patients the reserve for production of blood cells may be distinctly lower than in the normal If this is true the inclusion of even small amounts of hematopoietic tissue in the radiated field in patients receiving radiation therapy would be of much greater significance than ordinarily considered since most of such patients are suffering from malignancy or some other serious disorder which is associated with debilitation As a further indication that the effect may be due to inclusion of hematopoietic tissue in the radiated field is the well established fact that the leukopenia practically never results from radiation of the head in which case radiation can be given without inclusion of more than minimal amounts of active bone marrow Second a specific indirect effect of radiation may exist i e some substance or substances may be produced directly and characteristically by radiation and then transmitted to parts of the individual that were not exposed to radiation Our experiments have failed to reveal such a situation Third a nonspecific effect of radiation may occur that is radiation over a local area may produce certain nonspecific changes in the tissues exposed As a result of these nonspecific effects histological changes may develop in some unexposed tissues and leukopenia possibly be produced also It is again possible here that the nonspecific effects may be greater in a debilitated than in a normal individual Our experiments have failed to show the presence of any nonspecific substance capable of producing leukopenia We have not examined the tissues histologically but feel that the evidence in the literature is sufficient to justify the assumption that these nonspecific histologic changes did occur at least in the normal animals which were being cross circulated at the time their partner was irradiated

Substantial involution of lymphoid tissue not itself directly injured has been frequently observed as a nonspecific response to a wide variety of injurious agents Bardeen[2] in studying visceral changes occurring in patients dying of superficial burns noted striking histologic changes of a degenerative nature in lymphoid tissues and commented on the similarity of these changes to those found experimentally after injection of ricin or diphtheria toxin These widespread alterations in all the lymphoid organs of the body following burns have been amply confirmed by other investigators [5, 37, 3] Similar changes and transient leukopenia and lymphopenia have been observed in mice subjected to dry heat in nonfatal exposures [30, 33] Selye[43, 44, 45] in what he has chosen to term the alarm reaction has described striking involutionary and degenerative changes in all the lymphatic organs as a nonspecific response to a variety of insults (cold heat surgical shock drugs) Nonspecific damage apparently may result in atrophy of the spleen beginning in the center of the Malpighian corpuscles marked loss of weight of thymus and lymph nodes and at times even complete disappearance of germinal centers in the latter Zechwer[57] noted similar changes after injuring subcutaneous tissues by injection of formalin It is interesting that this involution of lymphoid structures does not occur in the adrenalectomized animal—particularly in the light

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Material

The material which we have ourselves intimately studied is based on 24 women with subacute and chronic infectious hepatitis of the type described above Their ages varied from 40 to 80 years The history of the disease when the investigation

Table 1.—Blood Findings in 24 Cases of Infectious Subacute and Chronic Atrophy of the Liver

No	Name	Age	Sex	Duration of disease (months)	Icteric index	Hemoglobin (Sahli) %	Red cells (millions)	Color index	Mean diameter (μ)	Takata-Ara	Sedimentation rate	Ascites, edema
1	A K L	5	♀	9	21	8	3.53	1.11	7.90	+++		+
	O P	70	♀	3	36	95	4.24	1.02	7.78	+	9	−
3	M M A	66	♀	15	117	69	3.36	1.03	8.01	+++	62	+
4	O O K	5	♀	13	15	71	3.02	1.19	8.15	+++	27	+
5	K K H	0	♀	3	10	91	3.95	1.15	49	+++	30	−
6	E P P	68	♀	4	30	83	3.65	1.14	7.95	+++		−
7	A H	62	♀	3	−	63	2.59	1.11	7.69	+		−
8	K N H	66	♀	1 (?)	12	93	4.65	1.00	7.7	+++	24	−
9	U M	51	♀	10	2	57	4.12	1.05	86	++		−
10	E J P	60	♀	6	15	80	3.54	1.13	7.64	+++	85	−
11	N B C	74	♀	13	21	78	3.56	1.10	8.62	+++		+
12	M K A	48	♀	6	24	65	4.20	0.7	7.77	+++	30	−
13	A K	79	♀	4	6	4	3.02	1.23	8.21	+++	48	+++
14	E C E S	71	♀	5	21	8	3.93	1.1	8.61	+++	15	+
15	E P	75	♀	5 (8)	14	89	3.23	1.38	8.10	+++		+
16	S W O	68	♀	6	16	49	2.1	1.14	7.4		133	−
17	N M O N	56	♀	3	135	87	3.91	1.12	7.9	+++	82	−
18	H P	72	♀	?	81	96	3.86	1.24	8.01	+++	74	+
19	M A B	75	♀	12	2	95	3.91	1.22	8.10	+++	40	+
20	H M A	68	♀	?	15	87	4.26	1.02	8.19	+++	15	−
21	M F M C	63	♀	3	72	83	3.91	1.06	8.05	+++		−
22	A C	58	♀	5	11	76	3.21	1.19	8.04	++		+
23	A C C	80	♀	? (1 year)	5	90	2.93	1.36	9.00	+++	14	+
24	B M L	69	♀	6	9	105	4.27	1.23	8.24	+++		+

Notes *Case 1* Died 1 month later Autopsy subacute atrophy of the liver *Case 3* Died a few days later Autopsy subchronic atrophy of the liver *Case 12* Died 2 months later Autopsy chronic atrophy of the liver *Case 14* Died a few days later Autopsy chronic atrophy of the liver *Case 15* Died a few weeks later Autopsy chronic atrophy of the liver *Case 18* Died a few weeks later Autopsy chronic atrophy of the liver *Case 23* Died 1 month later Autopsy chronic atrophy of the liver

took place varied from 1–15 months duration In other words such an interval had elapsed since the jaundice was first noticed but as in a number of these patients the disease developed rather insidiously it had probably lasted as a rule longer than the specified period Some of the patients had ascites and edema at the time the investigations were made others developed these symptoms later Some died shortly after the investigation but most died later on

Besides the above material we have had access to other cases of the disease

BLOOD AND BONE MARROW IN INFECTIVE SUBACUTE AND CHRONIC ATROPHY OF THE LIVER

By E. Meulengracht, M.D. and H. Gormsen, M.D.

Introduction

Numerous investigations have been made of the blood picture and bone marrow in hepatic diseases, particularly in cirrhosis of the liver. These have revealed that especially in cirrhosis of the liver macrocytic anemia frequently develops. This can be accepted as a well ascertained fact [4, 5, 1].

It has further been shown that in liver diseases, especially in cirrhosis, changes also occur in the bone marrow. One sees rather frequently a slight or moderate increase in the number of erythroblasts, possibly a shift to the left in the erythroblasts, often a fair number of large forms, but no megaloblastic change in the marrow. There is also some increase in the plasma cells and possibly also in the reticulum cells, with or without pigment. More rarely a slight myeloid hyperplasia or a slight eosinophilia are observed [6, 7, 1, 12, 15, 0].

As numerous cases of a special fatal form of subacute and chronic infective hepatitis have occurred in recent years and continue to do so here in Denmark, we have had the opportunity of examining the blood and bone marrow in this particular form of liver disease. The following notes deal with the nature of these cases.

In 1943 and 1944 subacute and chronic hepatitis began to occur in our clinic and in all other clinics in Denmark. Appearing suddenly and so extensively and with such malignant symptoms, it produced the impression that an entirely new disease had arisen, and soon came to be known among both doctors and laity as "the malignant and dangerous jaundice." The most striking fact is that the patients were almost exclusively women of from 40 to 70 years of age.

The clinical picture is dominated in these cases by long continued jaundice. It is as a rule only slight, but can be more intense or even severe. It varies somewhat in intensity with a tendency gradually to subside, but in its place ascites and edema make their appearance in many cases in a few months' time, though usually in six months or a year, frequently at the stage when the jaundice has greatly decreased. After a longer or shorter interval hepatic failure and death finally supervene. The prognosis is very grave, almost all the patients dying of the disease sooner or later.

At autopsy a small contracted liver with isolated remains of liver tissue and an extensive development of coarse connective tissue is found, a picture previously described [2] among others in 1930 during a Swedish epidemic.

Preliminary reports have been published on the numerous cases [1, 3, 9, 10] and a more comprehensive joint report will appear in the near future. The disease must presumably be regarded as a form of acute infectious hepatitis, the extensive epidemic of which in Denmark reached its climax in 1944.

From Medical Dept. B, Bispebjerg Hospital, Copenhagen, Denmark

cent Hb and between 3 and 4 million red cells The color indices are grouped around 1 1

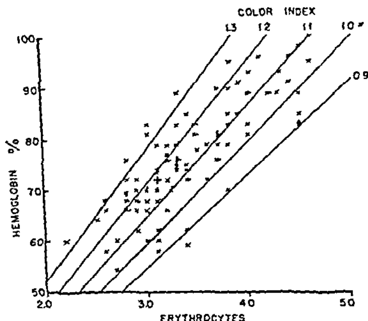


FIG 1 HEMOGLOBIN RED CELLS AND COLOR INDEX IN 141 DETERMINATIONS IN 75 CASES OF INFECTIVE SUBACUTE AND CHRONIC ATROPHY OF THE LIVER

Lines are continuations from a common point representing zero for both hemoglobin and erythrocytes

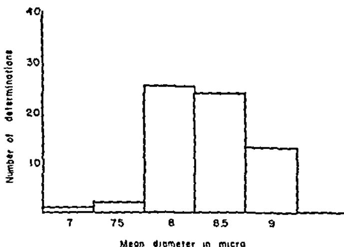


FIG 2 MEAN DIAMETER OF RED CELLS IN 64 MEASUREMENTS IN 52 CASES OF INFECTIVE SUBACUTE AND CHRONIC ATROPHY OF THE LIVER

In the closely studied cases the mean diameter of the red blood cells varied from 7 64μ to 9 0μ From the other material we collected 64 measurements in 52 patients there was about the same variation The values are given in table 2 and figure 2

approaching 150 in all and from their records we have been able to collect further information about the blood changes

Methods

All the blood examinations were made by two well trained technicians

The icteric index was determined by Meulengracht s m thod The hemoglobin was estimated in an Autenrieth Kønig berger colorimeter standardized to 100 per cent = 18 5 p r cent oxygen binding capacity determined by Van Slyke s method

Dilution of the blood for counting was done according to Ellermann s principle with separate pipets and mixing tubes the blood corpuscles were enumerated in Zeiss counting chamb rs The color index determinations were reckoned on the assumption that 100 per cent Hb corresponds to 5 000 000 red blood cells With these methods and standards we find the color ind x in normal p rsons to b about 1 1

The mean diameter of the red blood cells was determined by Gram s method where they are measured in their own serum so that one can b sure of avoiding the chang s which may occur on drying or washing in foreign media A drop of blood is sucked up by capillary attraction into a 5–10 cm long thin glass tube (capillary tube of about 1 mm thickness) The tube is s aled at both ends and put aside for some hours The blood has then clotted and the serum has separated at the side of the clot In this serum free blood corpuscles are present in sufficient quantities for measurement and in suitable numbers so that measurement is not imp-ded by the corpuscles lying too close to one another After the ends have been broken off the tube a small drop of serum is blown out into a counting chamber of half the usual height and is covered with a thin cover glass The measurements are made with th help of an ocular micrometer and immersion lens The diameters of 100 blood corpuscles at random are measured and the mean diameter calculated Mulberry shaped spherical and other abnormally shaped blood cells are avoided

The Takata reaction was performed by Jezler s modification The blood sedimentation rate was determined by Westergren s method

In the smear preparations of the sternal punctures a differential count of 200 cells was made Furthermore in every case a section of the coagulum of th sternal puncture after emb dding in paraffin was examined

The investigations of the sternal punctures were all made by one of us (H G)

Results

Blood In table 1 the age sex duration of the disease and the results of the blood examinations are given for those patients we ourselves thoroughly investigated

The icteric index varied from 7 to 135 it often became decreased as the cirrhosis progressed The Takata reaction in most of the patients was strongly positive (+++) but in some it was weaker (+ or ++) In one case it was negative but it is possible that the diagnosis was wrong The blood sedimentation rate was increased in all the cases investigated

The hemoglobin values varied from 50 to 105 per cent red blood cells from 2 17 millions to 4 65 millions In most cases there was some anemia The color index varied from 0 8 to 1 36 It was usually around 1 1 which with our standards corresponds to the normal in a few cases it was perceptibly diminished On microscopic examination of the stained dry preparation neither oval cells nor large ones of the nature of megalocytes were observed From the other more extensive material we have had access to we have collected 141 determinations in 75 patients Hb varied from 58 to 104 per cent R B C from 2 5 millions to 5 1 millions color index from 0 8 to 1 34 In figure 1 the Hb values are plotted against the red cell values and the color index given It will be seen that there is a marked tendency to a moderate degree of anemia the bulk of the values lying between 60 and 80 per

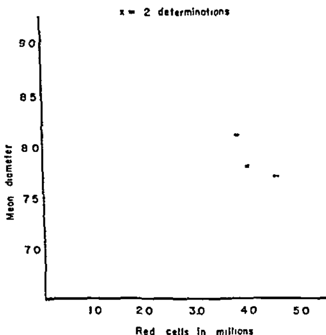


FIG 4 MEAN DIAMETER PLOTTED AGAINST RED CELLS

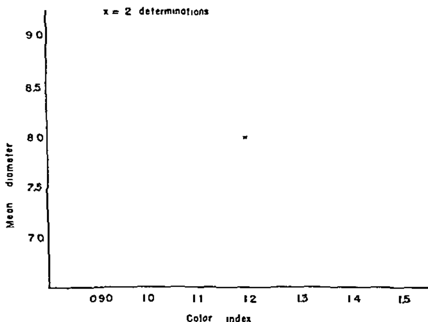


FIG 5 MEAN DIAMETER PLOTTED AGAINST COLOR INDEX

The blood platelets were counted in only a few of the cases There appeared to be a tendency towards low values

It will be seen that over 50 per cent lie above 8 μ With the same technic Gram[8] found values fluctuating between 7 7 μ and 8 0 μ in normal persons Jørgensen and Warburg[11] found roughly corresponding limits In our laboratory also the normal values found are almost identical with those of Gram On the basis of these facts it can be asserted that there is a very decided tendency towards an increase in the *mean diameter of the red cells in patients suffering from subacute or chronic atrophy* of the liver in No 23 it reached as much as 9 μ

TABLE 2 —*Mean Diameter of Red Blood Cells in 64 Measurements in 52 Patients*

μ	7	7 7 5	7 5-8	8 8 5	8 5 9
Number	1	2	35	23	13

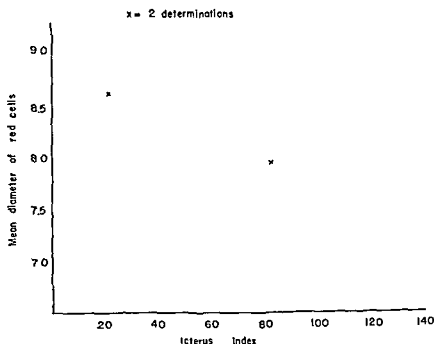


FIG 3 MEAN DI METER PLOTTED AGAINST ICTERIC INDEX

In figure 3 the mean diameter is plotted against the icteric index It will be observed that there is no correlation between the two

In figure 4 the mean diameter is plotted against the number of red cells There is no correlation between the mean diameter and the degree of anemia

In figure 5 the mean diameter is plotted against the color index Again no correlation can be detected

The number of white blood corpuscles varied between 1700 and 12,700 in 129 counts in 81 patients (table 3 and figure 6) The last value was quite an isolated one There was a distinct *tendency to low counts over 50 per cent of them being* under 4000 Unfortunately differential counts were done in only a few cases but as far as they went no definite deviation from the normal was found

forms so that the state of the marrow seemed to point to a slight increase in the functioning of the bone marrow with greatest emphasis on erythropoiesis

TABLE 4—*Res lts of Sternal Punctur n 24 Cases of Infectiie Subacute and Chronic At ophy of the Liier*

	N m l lue		No f pat t											
	%	%	1	2	3	4	5	6	7	8	9	10	11	12
Neutroph segment	19	10 –25	18	13	8	12	19	18	20	18	24	22	27	36
Neutroph nonsegment	6	2 –10	4	5	6	6	6	5	5	8	6	6	5	5
Neutroph metamyelocytes	15	8 –20	14	13	21	16	21	16	19	14	18	17	12	10
Neutroph myelocytes	12	[illegible] –18	9	11	13	15	12	11	12	16	13	11	11	9
Neutroph promyelocytes	4	2 – 7	5	6	–	6	6	6	6	6	6	5	4	3
Eosinoph matures	1	0 – 3 5	1	1	1	2	1	1		3	2	2	1	1
Eosinoph immatures	2	0 5– 4	2	5	4	1	4	3	3	4	4	2	1	2
Basoph granulat	1													
Hemocytoblasts	1	0 3– 2 5			1	1				1			1	
Lymphocytes	15	5 –25	11	8	4	11	8	10	12	6	9	10	18	12
Monocytes	1	0 – 3												
Plasma cells	1	0 – 3	3	3	3	2	1	2	0 5	2	1	1	0 5	2
Normoblasts	10	6 –17	21	17	19	13	9	15	10	12	6	11	10	9
Polychrome erythroblasts	6	3 – 9	6	10	7	9	7	7	7	6	5	7	4	5
Basophile *erythroblasts*	3	1 – 4	4	5	4	4	2	4	3	2	3	3	2	3
Megakaryocytes	1													
Reti ulum cells	3	0 5– 9	2	3	2	2	4	2	2 5	2	3	2	3 5	3

	Norm l alu		No of pat e t											
	%	%	13	14	15	16	17	18	19	20	21	22	23	24
Neutroph segment	19	10 –25	16	18	14	12	13	17	15	18	32	9	19	15
Neutroph nonsegment	6	2 –10	5	[illegible]	7	7	5	8	7	7	6	4	5	6
Neutroph metamyelocytes	15	8 –20	16	16	15	19	16	15	15	14	9	18	14	18
Neutroph myelocytes	12	7 –18	13	12	14	13	14	11	12	11	7	10	13	13
Neutroph promyelocytes	4	2 – 7	6	6	6	6	7	5	6	6	5	5	7	7
Eosinoph matures	1	0 – 3 5	1	2	1	1	1	1	1	1	1	1	1	1
Eosinoph immatures	2	0 5– 4	2	3	3	2	2	2	2	3	2	2	2	2
Basoph granulat	1													
Hemocytoblasts	1	0 3– 2 5	1					0 5	1				1	1
Lymphocytes	15	5 –25	8	9	12	12	10	15	13	16	22	9	21	15
Monocytes	1	0 – 3												
Plasma cells	1	0 – 3	3	1	2	1	4	4	3	2	2	3	2	4
Normoblasts	10	6 –17	15	11	15	15	13	11	10	9	6	9	7	9
Polychrome erythroblasts	6	3 – 9	7	8	7	6	7	5	7	6	4	13	4	4
Basophile erythroblasts	3	1 – 4	4	4	4	3	5	2	5	4	2	14	2	2
Megakaryocytes	1													
Reti ulum cells	3	0 5– 9	3	3	4	4	3	3 5	3	3	2	3	2	3

COMMENT

In the blood examinations i e determination of Hb per cent R B C color index and direct microscopy no changes such as those found in pernicious anemia

The bone marrow The results of the sternal punctures are given in table 4 As a basis for comparison the normal values found by Gormsen[7] in a material of 50 normal adults are given in the first column

A comparison with the normal values indicates that in the neutrophile granular cell series there were no changes no shift to the left no myeloid hyperplasia and no eosinophilia Hemocytoblasts lymphocytes megakaryocytes and reticulum cells exhibited normal appearances but there were certain abnormalities in the erythroblasts and plasma cells

Four patients had over 30 per cent erythroblasts 8 patients had from 22 to 26 per cent while the remaining 12 therefore half the patients had below 20 per cent

TABLE 3 —*Number of Leukocytes in 129 Counts in 81 Patients*

Leukocytes	1000–2000	2000–3000	3000–4000	4000–5000	5000–6000	6000–7000	7000–8000	8000–9000	9000
Number	3	25	44	24	23	4	4	1	1

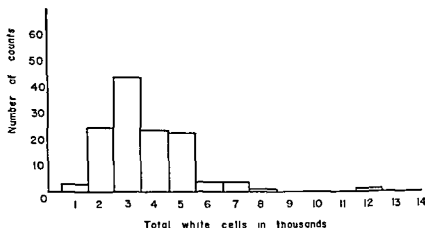


FIG 6 NUMBER OF WHITE CELLS IN 129 COUNTS IN 81 CASES OF INFECTIVE SUBACUTE AND CHRONIC ATROPHY OF THE LIVER

In none was the number of erythroblasts found to be lowered In one patient only (No 22) was there a shift to the left an the erythroblasts but it was very pronouced No definite macrocytosis of the erythroblasts could be detected in any of the cases Micrometric investigations of the size of the erythroblasts which normally vary considerably were not undertaken Megaloblasts were not observed in any of the cases

With regard to the plasma cells they were 4 per cent in 3 patients 3 per cent in 6 and below 3 per cent in the other 15 that is to say in 9 of the patients there was a slight increase in the plasma cells since the maximal value of 3 per cent for the normal number of plasma cells is very high

Microscopic sections of the bone marrow in 8 patients (Nos 1 2 3 8 11 12 17 18) showed slight to moderate hyperplasia of the marrow but in the others the cell content was normal The hyperplasia seemed to depend in part on a striking increase in the erythroblasts and also on a slight augmentation of all the other cell

blastic erythropoiesis but possibly to the serum content in substances which affect the osmotic condition of the blood corpuscles

The plasma cell increase in the bone marrow that was found in some of the cases is presumably connected with the hyperglobulinemia which is a very common accompaniment of chronic hepatitis

The blood and bone marrow changes in our patients thus do not seem to differ from the blood and marrow changes hitherto recognized in chronic hepatitis and liver cirrhosis

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were observed In conformity with this no sign of megaloblastic transformation of the red bone marrow was seen in any of the bone marrow punctures We have in some cases attempted to influence the anemia by liver injections but there was no reticulocyte reaction and no rise in the number of red blood cells

On the other hand a marked tendency to an increase in the mean diameter of the red blood cells was observed which in a few cases assumed very high proportions Gram [8] in 1883, in his measurements of the diameter of the red cells in various diseases demonstrated that the mean diameter in ikterus catarrhalis that is hepatitis is increased Determined in their own serum the values lay between 8 μ and 8 5 μ in one case with cirrhotic changes it was 8 9 μ This finding was later confirmed by others[11 14] using the same method The increase in the mean diameter in such cases of liver disease has been attributed to changes in the plasma possibly to the presence of the salts of bile acids The fact that in our bone marrow investigations only doubtful macrocytosis of the erythroblasts was demonstrated and at all events no megaloblastic change in the bone marrow seems to support the theory that the macrocytosis may at least partly be due to a change in the plasma with consequent swelling of the blood corpuscles Since there is no correlation between the degree of jaundice and the incrase in the mean diameter the latter cannot depend upon the bilirubinemia but must be caused by other substances in the plasma (bile acid salts?) The question needs a more elaborate investigation

The tendency to an increase in the plasma cells of the bone marrow must undoubtedly be connected with the plasma protein changes causing the positive Takata reaction which are characterized by an alteration in the albumin/globulin ratio decrease in the albumin fraction and increase in the globulin fraction

Conclusions

In the observed cases of subacute and chronic infectious atrophy of the liver of the special type which occurs at the present time in Denmark a moderate degree of anemia of a hypochromic normochromic or hyperchromic type was found in the majority of the cases The color index showed a fairly uniform distribution around the normal value 1 1 There was nothing in the blood picture that resembled true pernicious anemia

Measurements of red blood cells in their own serum revealed an increase in the mean diameter often considerable in over half the cases

The blood picture with respect to the white corpuscles showed a pronounced tendency in the direction of leukopenia

In some of the cases sternal punctures showed the presence of a more or less advanced erythroblastosis but no really definite macrocytosis of the erythroblasts was observed and megaloblastic erythropoiesis occurred in none of the cases A slight increase in the plasma cells was seen in some cases Sections of the bone marrow in a number of cases showed moderate hyperplasia of the marrow

In no case either in the blood or bone marrow could changes be demonstrated which would suggest nonstorage of the material necessary for the maturation of the red blood cells The increase in the mean diameter of the red blood cells measured in their own serum which was observed in some cases is not due to megalo

and because of accompanying weakness she reported to her physician A diagnosis of cord cervicitis was made and she was advised to enter the hospital for study On July 28 1940 she was admitted to another hospital where a blood culture revealed Staphylococcus albus She was given sulfathiazole 1 gram every 4 hours beginning on August 1 to 194 The hospital record shows that the blood cultures became sterile and she was discharged on August 1 She continued to take sulfathiazol and on August 14 two weeks after the institution of chemotherapy she complained of photophobia lacrimation and soreness and redness in several old scars around the left shoulder Later a rash developed around the eyes and over the legs Because of these findings the drug was discontinued by her physician with subsequent disappearance of all symptoms

Because she continued to exhibit a low fever of about 99 2 F on September 11 1940 her physician again started sulfathiazole therapy At 10 a m and 2 p m she took 1 gram At 4 p m she developed a chill with a subsequent rise in temperature to 1 3 F No further sulfathiazole was ingested until the following day when the dosage was increased to 1 5 grams Again she took the drug at 10 a m and 2 p m but after the latter dose she developed a rigor the temperature rising to 104 F The next day she complained of a severe headache aching in the joints nausea and vomiting and noticed that her urine had become quite dark in color She was hospitalized because it was thought she had developed hematuria

The patient gave a history of tonsillitis in 1936 for which she had received sulfanilamide without the development of toxic symptoms

On admission to the hospital the oral temperature was 100 F There was a definite icteric tint to the skin and sclerae There were no petechiae and no rash The vessels of the conjunctiva and sclerae were injected The nasal and pharyngeal mucous membranes appeared normal The lungs were clear to percussion and auscultation The heart was normal in size no murmurs were heard The liver edge was tender and extended 2 centimeters below the costal margin The spleen was not felt

Laboratory examinations included a total erythrocyte count of 4 000 000 per cubic millimeter a hemoglobin of 1 per cent and 4 200 leukocytes per cubic millimeter The differential count on the blood smear showed 68 per cent neutrophils 21 per cent lymphocytes 2 per cent monocytes and 9 per cent eosinophils There was bile in the urine as well as a trace of albumin

Clinical course The patient improved rapidly during the period of hospitalization The icterus index which was 25 on September 16 1940 had fallen to 4 on September 24 Bile was not detected in the urine after the third day in the hospital and the urobilinogen which was present in a 1 20 dilution decreased so that it was found only in undiluted urine The reticulocyte count was 0 6 per cent on September 18 and the erythrocyte count and hemoglobin concentration did not change significantly The Takata Ara test was positive and there was slight depression in the hippuric acid excretion test

Because it was felt that this patient's illness was caused by sulfathiazole she was given 0 5 gram on September 24 1940 One hour later she developed a rigor the vessels of the conjunctiva and sclera became congested she vomited twice and complained of headache and soreness in the scars on the left shoulder Four hours after the ingestion of the test dose of sulfathiazole the temperature reached 105 F She was then given an intravenous infusion of 1000 milliliters of saline and the temperature rapidly returned to normal No bile was detected in any of the urine specimens but the icteric index gradually increased to 10 during the subsequent 24 hours Urobilinogen was found again in dilutions of 1 20

To summarize this case the patient received an initial course of sulfathiazole and developed toxic symptoms two weeks later These symptoms included nausea vomiting episcleritis chills fever and skin rash The drug was discontinued for approximately seventeen days and upon its resumption immediate toxic effects were exhibited From the history it appears that jaundice developed two days after the first dose of the second course of sulfathiazole When she had recovered a test dose of 0 5 gram of sulfathiazole was administered Again she developed fever nausea vomiting and episcleritis The icterus index rose from 4 to 10 and remained elevated for forty eight hours This patient's illness is an example of immediate jaundice due to previous sensitization to the sulfonamide drugs There was little

JAUNDICE AND THE SULFONAMIDE DRUGS

By Charles H Rammelkamp M D

IT IS NOW well established that jaundice is one of the toxic manifestations of sulfonamide chemotherapy Although it is usually possible to recognize this complication sulfonamide induced jaundice may be difficult to differentiate from toxic hepatitis occurring as a complication of the infectious process itself as well as from sporadic instances of infectious hepatitis and serum jaundice Since the sulfonamide drugs are widely used in medical practice it is perhaps of some interest to review the distinguishing characteristics of toxic hepatitis caused by these compounds The establishment of a definite diagnosis in jaundiced patients is important because all sulfonamides should be discontinued immediately if this form of chemotherapy appears to be responsible for the toxic complication In individuals with jaundice due to other causes sulfonamide medication especially sulfadiazine may be given without causing further damage to the liver parenchyma [1]

In general three forms of jaundice may accompany the use of the sulfonamide drugs They may be classified as immediate intermediate and delayed depending upon the time of appearance of toxic symptoms including icterus *following the* initiation of therapy

Immediate Jaundice

The immediate form of toxic hepatitis secondary to sulfonamide medication is usually readily recognized Within a period of one to three days from the time chemotherapy is started jaundice appears Prior to this however and usually within a few hours after the initial dose of sulfonamide other toxic symptoms appear These include nausea vomiting headache chills fever burning of the eyes and skin rashes As the drug is continued the skin may become icteric and the urine dark with bile Examination usually reveals an enlarged tender liver as well as various forms of skin rashes The erythrocyte count and hemoglobin concentration are usually normal and the reticulocyte count is not elevated The leukocyte count may be elevated or normal

These patients give a history of previous ingestion of one of the sulfonamide drugs and usually state that toxic symptoms occurred at that time [2] An example of this form of hepatitis is reported below

Case Report

A 45 year old married woman entered the Evans Memorial Massachusetts Memorial Hospitals on September 14 1940 because of chills fever and nausea of three days duration About eight month prior to admission she noticed that her afternoon oral temperature occasionally reached a high a 99 6 F

From the Departments of Preventive Medicine and of Medicine Lakeside Hospital Western Reserve University Cleveland Ohio

* I an indebted to Dr Chester S Keefer for permission to report this cas

a rash which may be erythematous or at times exfoliative in character Erythema nodosum is not infrequently observed in patients treated with the thiazole derivatives of the sulfonamides Jaundice develops within a few hours or days after these other toxic symptoms have become manifest

The physical signs and abnormalities in the laboratory examinations are similar to those found in patients developing the immediate form of jaundice The liver and spleen may be enlarged and occasionally ascites may develop There may be a moderate anemia especially if the reaction occurs after the ingestion of sulfanilamide Usually there is an increase in the total leukocyte count which may be marked when there is an extensive rash and occasionally there may be leukopenia The bilirubin content of the blood is increased and urobilinogen and bile are found in the urine Impairment of liver function may be demonstrated by several tests

Although most patients receiving sulfonamide therapy and developing the delayed form of jaundice conform to the above description there are a few in whom the diagnosis is difficult Toxic hepatitis apparently may develop several weeks after the cessation of chemotherapy Garvin[2] reports an instance where jaundice and exfoliative dermatitis developed forty three days after all sulfonamide medication had been stopped In some patients jaundice appears as the only toxic manifestation of chemotherapy One patient developed jaundice sixteen days after sulfanilamide therapy was instituted and no other symptoms were recorded In this instance the patient was being treated for chronic prostatitis so that it would appear improbable that the hepatitis was secondary to the infectious process

Discussion and Summary

It is apparent from a review of the reported cases of hepatitis associated with sulfonamide therapy that it is usually possible to recognize this toxic manifestation This is of considerable practical importance since in every instance in which the sulfonamide is responsible for the jaundice treatment with the drug should be discontinued and some other form of therapy such as the antibiotics instituted If the jaundice is not secondary to the sulfonamide therapy may be continued even in the presence of hepatitis secondary to the infection[1]

Jaundice which appears during the first week of chemotherapy is usually associated with a previous history of ingestion of sulfonamides and accompanying signs of toxicity (immediate sulfonamide jaundice) or with acute hemolytic anemia and jaundice (intermediate jaundice) In either instance the diagnosis is not difficult because the clinical and laboratory abnormalities are characteristic Finally jaundice which occurs after ten days of chemotherapy is usually associated with other toxic manifestations especially fever and various forms of rashes (delayed jaundice) Occasionally jaundice may be the only toxic manifestation of sulfonamide therapy and in such patients a definite diagnosis may be difficult

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evidence of hemolytic anemia although from the studies recorded it is not possible to state that *some increase in the rate of destruction of the erythrocytes* did not occur

Intermediate Jaundice

This form of jaundice is associated with a mortality rate of 5 to 10 per cent[3] and is readily diagnosed as sulfonamide induced since it is secondary to acute hemolytic anemia as well as to toxic hepatitis The anemia usually becomes prominent enough to cause pallor weakness dyspnea and nausea and vomiting in about two to five days after the institution of therapy [4] although occasionally it may occur later [5, 6] Soon after the development of the acute hemolytic anemia jaundice may appear This is believed to be due not only to the very great destruction of erythrocytes but also to some direct action on the liver cells [7]

The clinical features of this form of jaundice are readily recognized The patient invariably becomes critically ill during a period of a few hours pallor is marked the liver and spleen may be enlarged and fever is usually present Hemoglobin may appear in the urine Later biliuria and urobilinogenuria are observed The erythrocyte count and hemoglobin concentration are low and a smear of the blood may show nucleated erythrocytes as well as variations in the size and shape of the cells The reticulocyte count becomes markedly elevated There may be spherocytosis and an increased hypotonic fragility during the acute phase of the disease [3]

The total leukocyte count is usually markedly elevated counts of 100 000 per cubic millimeter not being unusual Immature cells are observed as well as an increased number of eosinophils The blood usually contains free hemoglobin as well as increased amounts of bilirubin

It is apparent from this description that this form of sulfonamide jaundice should be easily recognized It occurs most frequently following sulfanilamide medication although sulfathiazole sulfapyridine and sulfadiazine occasionally cause acute hemolytic anemia and jaundice When such toxic reactions occur the drug must be stopped immediately and treatment including blood transfusion instituted

Delayed Jaundice

Jaundice may appear ten or more days after the institution of sulfonamide treatment *Under these circumstances the diagnosis of sulfonamide hepatitis may be difficult* since jaundice may develop during the same period as a complication of the infectious process itself Generally however in those patients whose jaundice is secondary to chemotherapy there are other associated toxic symptoms and physical signs [8]

It is the usual experience that this form of hepatitis develops at about the time clinical improvement is anticipated The temperature which may have been normal or somewhat elevated suddenly increases In some instances it may be septic in type and associated with severe chills Nausea vomiting and epigastric discomfort may be prominent Other symptoms of toxicity include headache dizziness photophobia and aching of the joints A distinctive feature which is of considerable aid in the establishment of the correct diagnosis is the appearance of

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THIAMIN DEFICIENCY IN THE RHESUS MONKEY

Clinical Metabolic and Hematologic Observations

By J F Rinehart M D L D Greenberg Ph D and L L Ginzton M D

AN IMPORTANT advance in nutritional research was the development of an essentially synthetic diet adequate for study of single deficiencies in the monkey [1] It seemed most timely to restudy the vitamin deficiencies in a primate whose metabolic processes might be expected to approximate those of man most closely This report is concerned with the study of thiamin deficiency Seven rhesus monkeys (Macaca mulatta) were subjected to one or more episodes of thiamin deficiency Observations were made on food consumption weight clinical behavior thiamin metabolism and the blood picture Finally the animals were sacrificed and detailed pathologic examinations were made In this report the experimental method is reported together with the clinical metabolic and hematologic observations enumerated above The pathologic findings resulting from recurrent thiamin depletion with particular reference to characteristic degenerative changes occurring in the heart muscle and severe retrogressive changes in the nuclear structures of the central nervous system have been reported [2] and will be detailed elsewhere

Experimental Method

The diet used in these experiments was a modification of the M 3 diet of Waisman et al[1] and consisted of powdered sucrose 73 vitamin test casein (General Biochemicals) 18 Hawk and Oser salt mixture 4 and corn oil 1 Sulfited liver extract equivalent to 100 grams of Wilson Laboratories L fraction prepared according to the method of Kline and co-workers[4] was added to each 4 kilograms of diet The diet was dried granulated and following the addition of 1 per cent calcium stearate compressed into tablets weighing approximately 2 grams The basal diet was fed ad libitum The diet in pellet form had the advantage of curtailing waste and facilitating the estimation of the daily food consumption A vitamin tablet containing daily dosages similar to those of Waisman et al was fed each day Each vitamin tablet contained the following nicotinic acid 5 mg riboflavin 1 mg pyridoxine hydrochloride 1 mg calcium pantothenate 3 mg choline dihydrogen citrate 100 mg paraminobenzoic acid 100 mg inositol 100 mg and ascorbic acid 25 mg plus sufficient powdered sugar to make a tablet weighing 1 5 to 2 grams The monkeys accepted the e v tamin tablets willingly and consumed them eagerly Control monkeys were also given 1 or 0 5 mg thiamin chloride daily In addition the monkeys received by mouth 5 drops of vitamin A and D concentrate twice weekly and additional sulfited liver extract (equivalent to 2 5 Gm daily) twice or thrice weekly as a source of biotin and folic acid Some animals also received 5 drops of mixed tocopherols (Napco) once a week

During the course of these studies the weights and daily food consumption were followed carefully Blood was taken by venipuncture at approximately weekly intervals for the determination of thiamin

From the Department of Pathology University of California Medical School San Francisco California

The work has been aided by a Grant from the California Fruit Growers Exchange and the A B Miller Fund We are indebted to David Klein of Wilson Laboratories for generous supplies of Liver Fraction L

We are grateful to Mr S J Dean of the College of Pharmacy for his assistance in the preparation of these tablets

levels and for hematological studies At autopsy portions of several tissues were removed from each monkey and prepared for analysis of thiamin and riboflavin content The methods used for the analysis of thiamin of blood and tissues have previously been described [8] The method for the estimation of riboflavin will be reported in another article The animals were tuberculin tested by injection with old tuberculin in the eyelids Positive reactors were rejected The monkeys were then placed on the purified diet with complete supplementation for one to two weeks and control tests were carried out so that each monkey could serve as his own control The occasional animal who failed to adapt itself to the diet or failed to gain in weight during the control period was rejected With the exception of one monkey all the animals used weighed between 1800 and 3600 grams The one exception was an older animal weighing 7000 grams which had previously b en used in some other studies In all 7 monkeys were employed in this study and were subjected to one or more p riods of depletion

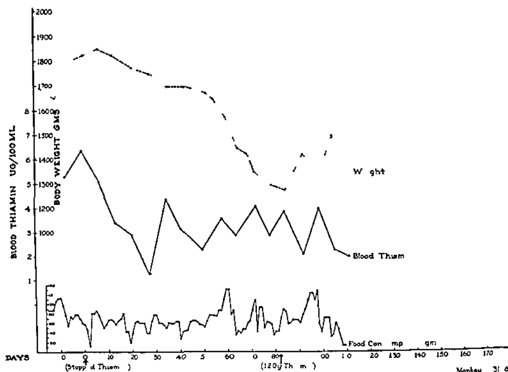


Fig 1 Thiamin Deficiency in Monkey No 3146
Graph of food consumption blood thiamin and weight

Clinical Observations

The clinical behavior of the animals was in most respects similar to that reported by Waisman and McCall [4] In general the monkeys ceased gaining after two weeks on the thiamin deficient diet This was either followed by a plateau of the weight curve for several days or by loss of weight The weight loss was usually associated with a decreased food consumption and marked lowering of the blood thiamin as is shown in figure 1 and 2 which are representative of the changes observed in monkeys on the thiamin deficient diet As the deficiency progressed the animals continued to lose weight became apathetic and inactive and weakness was evident Finally if the depletion period were prolonged the animals became ataxic Some developed ptosis and tremors Retching was observed on several

occasions The monkey would make every attempt to prevent the escape of vomitus from his mouth by trapping it in his buccal pouches and would ultimately reswallow it Convulsive movements have been observed in one or more of the animals If thiamin were administered at this stage a dramatic response was observed in twenty four to forty-eight hours The improvement in locomotion alertness and appetite was striking On the other hand if the period of thiamin deprivation were not interrupted at this stage it was but a matter of a few days until the monkey was unable to sit on its perch or even stand upon the floor of the cage without difficulty

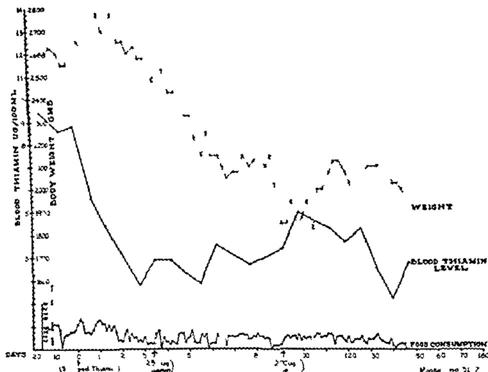


Fig 2 Thiamin Deficiency in Monkey No 3177
Graph of food consumption blood thiamin and weight

At times there appeared to be a paralysis of the hind legs The animal could climb or move in the cage only by the use of its forelegs Occasionally the onset of acute thiamin deficiency was so sudden that no manifestations were evident aside from weight loss mild anorexia and decreased activity until the animal became ataxic followed by a state of collapse Edema was observed in only one animal during the period of depletion However following administration of thiamin to acutely deficient animals we have in several instances observed the appearance of edema The control animals continued to gain weight (although not as rapidly as animals we have had on our stock diet) and to remain strong and healthy during the course of the experiment We have maintained control animals on the complete diet

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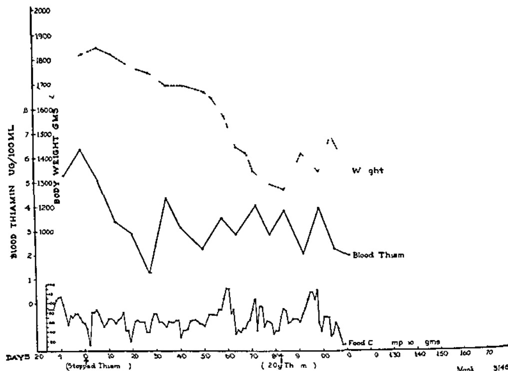


Fig 1 Thiamin Deficiency in Monkey No 3146
Graph of food consumption blood thiamin and weight

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The clinical behavior of the animals was in most respects similar to that reported by Waisman and McCall [4] In general the monkeys ceased gaining after two weeks on the thiamin deficient diet This was either followed by a plateau of the weight curve for several days or by loss of weight The weight loss was usually associated with a decreased food consumption and marked lowering of the blood thiamin as is shown in figure 1 and 2 which are representative of the changes observed in monkeys on the thiamin deficient diet As the deficiency progressed the animals continued to lose weight became apathetic and inactive and weakness was evident Finally if the depletion period were prolonged the animals became ataxic Some developed ptosis and tremors Retching was observed on several

value obtained is 15 5 micrograms per kilograms per day and is in close agreement with the value reported by Waisman and McCall [4] It should be pointed out that this does not represent an intake adequate for normal metabolism

Tissue thiamin Certain tissues were removed at the time of autopsy and subjected to thiamin and riboflavin analysis In nearly all cases (with the exception of one) the deficient animals were sacrificed in the terminal stage of deficiency by the administration of chloroform For purpose of comparison a control animal was sacrificed at the termination of the study The latter was in excellent health after having been maintained on this purified diet for a period of six months In table 2 we have summarized the results of our analyses These data show that there is a marked lowering of the thiamin content of every tissue examined in the deficient animals

In the case of riboflavin the heart kidney and liver concentrations of the deficient animals were found to be slightly higher than those observed in the control

TABLE 2

Monkey	Control (C) deficient (D)	Skeletal Muscle		Brain (cortex)		Heart (ventr)		Kidney		Liver	
		Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin
		γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm
3163	C	0 9	3 1	1 6	2 5	3 5	6 8	3 5	19 6	2 1	18 9
3175	D	0 4	—	0 3	—	0 3	6 6	0 5	17 8	0 7	24 1
3192	D	0 2	1 8	0 4	2 8	0 3	8 7	0 6	26 3	0 5	21 4
317	D	0 3	1 7	0 5	2 5	0 3	10 0	0 9	23 1	0 7	26 8
3146	D	0 5	1 8	—	—	0 4	10 2	0 6	22 0	0 6	25 4
69	D	0 2	1 5	—	—	0 9	9 6	1 0	24 8	0 8	21 4
73	D	0 2	1 2	—	—	0 3	7 3	1 3	21 3	0 9	18 5
245	D	0 3	1 2	—	—	0 3	8 2	0 8	25 2	0 9	18 4

animal However this is offset by the greater concentration of this vitamin in the skeletal muscle of the control animal

Although it is difficult to compare tissue vitamin levels of one species of mammal with those of another species since the intake is known definitely to influence the concentration of the vitamins we can point out that the control levels found in the monkey are similar in some respects to those found in the rat and in man Figures[6] obtained in our laboratory on the rat on a 20γ per day level are of the same order for heart and muscle but higher values for kidney and lower values for liver are found in the monkey In general rats with acute thiamin deficiency show lower figures for the tissues analyzed than those found in the monkey during the acute stage of deficiency The control thiamin values for monkey s skeletal muscle and heart are not significantly different from those of humans who have died of accidental death Liver and kidney of human origin have shown lower values than those found in monkeys The tissue thiamin values recorded in the control monkey probably represent saturation values in as much as the animal had received daily thiamin in excess of its metabolic requirement

for a year or longer without any obvious alterations in their strength or vigor One control animal was sacrificed and autopsied after having been on the diet for a period of six months and no pathologic changes were in evidence during either gross or microscopic examination of the tissues

Thiamin Metabolism

Blood thiamin During the period while the monkeys were on the complete diet including the thiamin supplement, the blood thiamin levels ranged from 5 5 to 10 micrograms per 100 ml of whole blood This range of values is similar to that observed by us in healthy human beings Following withdrawal of the thiamin and simultaneously with the first fall in weight and food consumption the blood thiamin usually dropped to values of 4 micrograms or less Except for some minor fluctuations these values remained low When sufficient thiamin was administered this was reflected in the blood level by a significant rise The alterations of the

Table 1 —*Thiamin Requirement of Monkeys*

Monkey no	Wt	Dose of thiamin administered	Elapsed time	Minimum requirement micrograms kg /day
	kg	*µg*		
245	5	4000	36	22
69	2 75	1000	50	7 5
3192				
1st–2nd depletion	1 9	200	11	9 6
2nd–3rd depletion	1 7	500	12	24 6
3rd–4th depletion	1 7	250	8	18 3
3	2 57	2500	91	27 5
Average				15 5

blood thiamin levels are charted in figures 1 and 2 The blood thiamin levels of control animals remained well above 5 mg per 100 ml during the course of the experiment

Minimal thiamin requirement A rough estimate of the minimum thiamin requirement of the monkey can be obtained by observing the time required to redeplete an acutely deficient animal following the administration of a small dose of the vitamin Although there is a possibility that a portion of the vitamin may pass through the gastrointestinal tract unabsorbed (if administered by mouth) or that a portion may be eliminated in the urine if the dose is too large for this calculation the assumption is made that the full dose is retained If the total dose is divided by the product of the elapsed time in days and the weight in kilograms one obtains a value for the minimum daily requirement per kilogram of body weight Calculations of this type have been carried out on 4 individual monkeys and are summarized in table 1 In the case of Monkey No 3192 which was carried through four depletion periods three different observations on this same animal are recorded The values recorded show considerable variation However the average

value obtained is 15.5 micrograms per kilograms per day and is in close agreement with the value reported by Waisman and McCall.[4] It should be pointed out that this does not represent an intake adequate for normal metabolism.

Tissue thiamin. Certain tissues were removed at the time of autopsy and subjected to thiamin and riboflavin analysis. *In nearly all cases (with the exception of one)* the deficient animals were sacrificed in the terminal stage of deficiency by the administration of chloroform. For purpose of comparison a control animal was sacrificed at the termination of the study. The latter was in excellent health after having been maintained on this purified diet for a period of six months. In table 2 we have summarized the results of our analyses. These data show that there is a marked lowering of the thiamin content of every tissue examined in the deficient animals.

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TABLE 2

Monkey no.	Control (C) or deficient (D)	Skeletal Muscle: Thiamin	Skeletal Muscle: Riboflavin	Brain (cortex): Thiamin	Brain (cortex): Riboflavin	Heart (ventricle): Thiamin	Heart (ventricle): Riboflavin	Kidney: Thiamin	Kidney: Riboflavin	Liver: Thiamin	Liver: Riboflavin
		γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm	γ/gm
3163	C	0.9	3.1	1.6	2.5	3.3	6.9	3.5	19.6	2.1	19.9
31[illegible]5	D	0.4	—	0.3		0.3	6.6	0.3	1[illegible].8	0.[illegible]	24.1
319[illegible]	D	0.2	1.9	0.4	2.8	0.3	8	0.6	26.3	0.5	21.4
3[illegible]	D	0.3	1.[illegible]	0.5	2.5	0.3	10.0	0.9	23.1	0	26.8
3146	D	0.5	1.8	—	—	0.4	10.2	0.6	22.0	0.6	25.4
[illegible]69	D	0.2	1.5	—	—	0.9	9.6	1.0	24.9	0.8	21.4
[illegible]3	D	0.[illegible]	1.2	—		0.3	[illegible].3	1.3	21.3	0.9	18.5
[illegible]245	D	0.3	1.2	—	—	0.3	9.2	0.8	25.2	0.9	18.4

animal. However, this is offset by the greater concentration of this vitamin in the skeletal muscle of the control animal.

Although it is difficult to compare tissue vitamin levels of one species of mammal with those of another species since the intake is known definitely to influence the concentration of the vitamins, we can point out that the control levels found in the monkey are similar in some respects to those found in the rat and in man. Figures[6] obtained in our laboratory on the rat on a 20γ per day level are of the same order for heart and muscle, but higher values for kidney and lower values for liver are found in the monkey. In general, rats with acute thiamin deficiency show lower figures for the tissues analyzed than those found in the monkey during the acute stage of deficiency. The control thiamin values for monkey's skeletal muscle and heart are not significantly different from those of humans who have died of accidental death. Liver and kidney of human origin have shown lower values than those found in monkeys. The tissue thiamin values recorded in the control monkey probably represent saturation values in as much as the animal had received daily thiamin in excess of its metabolic requirement.

HEMATOLOGIC OBSERVATIONS

It is generally considered that thiamin deficiency per se has little if any influence in hematopoiesis [7, 8] Our data indicate a significant influence on erythropoiesis as is illustrated in figure 3 It will be seen that with depletion for thirty days there was a slight but definite reduction in the red blood cell count and hemoglobin At forty days this was obscured presumably by dehydration However at this time the reticulocyte count had fallen to zero On administration of small subcurative doses of thiamin there were definite reticulocyte responses Interestingly this was accompanied by a fall in the red blood cell count and hemoglobin which was probably brought out by correction of dehydration A second

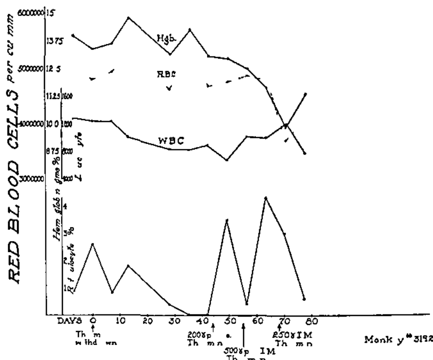


FIG 3 BLOOD IN THIAMIN DEFICIENCY IN MONKEY NO 3192

animal showed a progressive fall of the red blood cell count and hemoglobin beginning at thirty days and continuing to the termination of the experiment at fifty days In this animal the red blood cell count fell from 4 7 million to 3 6 million and the hemoglobin fell from 13 4 grams to 8 75 grams The reticulocyte count during the early phase of the experiment approximated 1 per cent falling to zero between thirty two and fifty days The complete suppression of circulating reticulocytes is of particular interest and is unique in our experience not occurring in other deficiencies of the vitamin B factors studied The small bleedings for metabolic studies probably contributed very little to the anemia Such anemias did not develop in control animals The cases cited are typical of the 4 animals in which hematologic examinations were made The conclusion seems justified that thiamin

deficiency in the rhesus monkey will cause anemia and that the mechanism is evidently due to suppression of reticulocyte formation *

SUMMARY

Seven rhesus monkeys were subjected to one or more episodes of acute thiamin depletion It is clear that significant metabolic inadequacies preceded demonstrable structural changes Diminished food consumption and weight loss were manifest about two weeks after thiamin was removed from the diet When the deficiency was prolonged the animals became apathetic inactive and progressively weaker This was followed by ataxia and at times ptosis and tremors Even in such advanced states of depletion administration of thiamin produced dramatic improvement in locomotion appetite and reactivity The blood thiamin content of normal monkeys ranged from 5 5 to 10γ per 100 ml of whole blood values which are comparable to those of healthy human beings Following withdrawal of thiamin the blood concentration fell to values of 4γ or less The tissue content of thiamin was correspondingly reduced in depleted animals The minimum daily requirement for thiamin calculated on the basis of the time required to redeplete a deficient monkey following a small dose of thiamin was approximately 15γ per kilogram body weight Characteristic degenerative changes in the heart muscle and severe retrogressive changes in the nuclear structures of the central nervous system previously reported were noted Based on careful hematologic studies in 4 animals it is concluded that thiamin is essential for normal erythropoiesis Acute or chronic depletion results in anemia due to suppression of red blood cell formation as indicated by severe depression or absence of reticulocytes in the blood

ACKNOWLEDGMENT

We are indebted to Miss Mariette Quigley for the blood counts and to Miss Ruth Johnson for assistance in thiamin assays

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* Since this article was written we have found a report by Fornaroli (Arch 6 ol 41 2 6-285 1941) in which h records a large reduction in r ticulocytes in vitamin B_1 deficient rats



AN
EXPERIMENTAL INQUIRY

INTO THE

PROPERTIES of the BLOOD

P R E F A C E

THE knowledge of the human frame, the preservation of health, and the cure of diseases are objects of too great importance to mankind, for the Author of these sheets to doubt, that any attempts to promote them, how small soever, should not meet with a candid and indulgent reception from the public An Inquiry into the Properties of the Blood, it is presumed will be thought, in a particular manner interesting since there is no part of the human body upon which more physiological reasoning is found

It is well known that the *crassam ntum* consists of two parts, of which one gives it solidity, and is by some called the fi brous part of the blood, or the *gluten*, but by others with more propriety termed the *coagulable lymph*; and of another, which gives the red colour to the blood, and is called the *red globules* These two parts can be separated by washing the *crassamentum* in water, the red particl s dissolving in the water, whilst the coagu lable lymph remains solid That it is the coagulable lymph, which, by its becom ing solid, gives firmness to the *crassamentum*, is proved by agitating fresh blood with a stick, so as to collect this substance on the stick in which case the rest of the blood remains fluid *

It may be proper to mention here that till of late the coagulable lymph has been confounded with the serum of the blood wh ch contains a substance that is likewise coagulable But in these papers by the *lymph* is al vays meant that part of the blood which jellies or b omes sol d spo ta neously when blood is received into a bason h h the coagulable matter that is d ssol ed in the serum does not; but agrees more with the wh te of an egg in remaining fluid when exposed to the a r a d coagulat g when expos d to h at or when m xed with ardent sp rits or some other chemical substances.

FIG 1 The Preface of Hewson s work An Experimental Inquiry into the Properties of the Blood his portrait and excerpts from pages 6 and 7 describing agitating fresh blood with a stick so as to collect fibrinogen

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THE CHEMICAL SPECIFICITY OF THE INTERACTION OF DIVERSE HUMAN PLASMA PROTEINS

By Edwin J Cohn Ph D

RECOGNITION of the specific chemical functions of the various protein components of the plasma began in the eighteenth century In his book An Experimental Inquiry into the Properties of the Blood published in 1771 Hewson (fig 1) described the separation of fibrinogen from plasma[1]

> When fresh blood is received into a bason and suffered to rest in a few minutes it jellies or coagulates and soon after separates into two parts distinguished by the names of *Crassamentum* and *Serum*
>
> It is well known that the *crassamentum* consists of two parts of which one gives it solidity and is by some called the fibrous part of the blood or the gluten but by others with more propriety termed the *coagulable lymph* and of another which gives the red colour to the blood and is called the *red globules* These two parts can be separated by washing the *crassamentum* in water the red particles dissolving in the water whilst the coagulable lymph remains solid That it is the coagulable lymph which by its becoming solid gives firmness to the *crassamentum* is proved by agitating fresh blood with a stick so as to collect this substance on the stick in which case the rest of the blood remains fluid
>
> It may be proper to mention here that till of late the coagulable lymph has been confounded with the serum of the blood which contains a substance that is likewise coagulable But in these papers by the *lymph* is always meant that part of the blood which jellies or becomes solid spontaneously when blood is received into a bason which the coagulable matter that is dissolved in the serum does not but agrees more with the white of an egg in remaining fluid when exposed to the air and coagulating when exposed to heat or when mixed with ardent spirits or some other chemical substances

Hewson thus recognized the water soluble constituent of the red globules the fibrinogen of the crassamentum or clot and the coagulable proteins largely the albumins of the serum Serum proteins insoluble in water at a slightly acid reaction but dissolved by salt were recognized by Denis and Scherer in 1841 Over a century ago therefore the chief protein component of the red cells and at least three protein components of plasma had been recognized

We now know that the pigment of the red globules largely responsible for the respiratory function of the blood is the prosthetic group of the protein hemoglobin and that the red blood cells contain in addition a large number of recently discovered protein components among them carbonic anhydrase catalase phosphatase choline esterase hypertensinase and other peptidases In this communication we shall concern ourselves however with the plasma proteins and the specific chemical reactions upon which their physiologic functions depend

The relation of fibrinogen to the clotting of the blood was implicit in Hewson s discovery Although Hewson found ways of blocking fibrin formation it remained

This paper is Number 63 in the series Studies on Plasma Proteins from the Harvard Medical School Boston Massachusetts on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross

for Andrew Buchanan to demonstrate in 1845 that fibrin has not the least tendency to deposit itself spontaneously in the form of a coagulum that like albumen and casein fibrin only coagulates under the influence of suitable reagents and that the blood and most other liquids of the body which appear to coagulate spontaneously only do so, in consequence of their containing at once fibrin and substances capable of reacting upon it and so occasioning coagulation [3] The substance which Buchanan noted by virtue of its physiologic action was presumably thrombin Satisfactory studies upon thrombin as a globulin were however not carried out until this century [4-9]

Meanwhile albumins and other globulins had been noted in terms of chemical properties often however without these properties being associated with specific biologic functions The chemical studies which have continued during the last century have yielded relatively pure preparations of albumins and demonstrated that there were many kinds of globulins

Molecular Dimensions of Plasma Proteins Although there is evidence of more than one albumin[10-15] all serum albumins which have been studied thus far of human or animal origin have closely similar molecular properties isoelectric points near pH 4 9 molecular weights near 70 000 and molecular dimensions described as ellipsoids 150 Angstroms in length and 38 Angstroms in width All plasma proteins thus far investigated have diameters of this magnitude or greater If the smallest dimension is less the molecule appears not to be retained in the blood stream but to be lost through the kidney Among molecules of the same diameter where loss occurs it appears to be inversely related to the length of the molecule This should apply not only to rod shaped molecules of diameters of the order of 20 Angstroms or less * [16] but also to the plasma proteins under pathologic conditions Among plasma proteins with roughly the same diameter of 38 Angstroms the β_1 globulins which combine and transport copper and iron have been estimated to have a length of 190 Angstroms the γ globulins concerned with immunity of 235 Angstroms and fibrinogen concerned with blood coagulation of 700 Angstroms [17]

The viscosity of proteins depends of course not upon their size but upon their asymmetry Thus fibrinogen the most asymmetrical of the plasma proteins has an intrinsic viscosity six times that of albumin Were it present in the plasma in large amounts instead of to but 4 per cent it would impose considerable burden upon the circulation Serum albumin is far more symmetrical a 25 per cent solution being isoviscous with blood [18 19]

The albumins are moreover the most stable the smallest and the most copious of the plasma proteins Present in normal plasma to just over 50 per cent they are responsible for nearly 80 per cent of the colloid osmotic pressure which regulates the equilibrium in water and electrolytes between the plasma and the tissues and thus play the major role in maintaining the volume of the blood upon which normal circulation depends [16 0]

The molecular size and shape of proteins once they are separated as homogenous

Characteristic of the arious suggested blood bstitutes h ch were examined but not recommended to the Armed Forces

chemical components may be estimated by measurements of osmotic pressure and of viscosity The ultracentrifuge renders it possible however to distinguish proteins of different molecular dimensions even in so complex a mixture as the plasma The constant defining the speed of motion of a protein in the field of the ultracentrifuge for the development of which we are indebted to Svedberg [1] has revealed proteins sedimenting in plasma with four very different velocities [1,11] Those sedimenting with the smallest velocity include the albumins and certain globulins The other globulins which have been separated and fibrinogen sediment with other velocities However ultracentrifugal analysis does not permit us to distinguish a large number of components in the plasma

Electrophoretic Mobilities of Plasma Proteins Solubility studies during the nineteenth century indicated that there were many globulins and classified them as euglobulin or pseudoglobulin depending upon their insolubility or solubility in the absence of salt or in concentrated salt solutions Electrophoretic analysis refined by Tiselius distinguished globulins in terms of their mobility in an electric field and designated them α β and γ globulins [5-7] Better resolution by the optical system employed in the analysis has revealed more than one α more than one β and more than one γ globulin Two α globulins α_1 and α two β globulins β_1 and β and two γ globulins γ_1 and γ the second one in animals sometimes termed a T globulin are now often designated If we include the results of electrophoretic analyses the number of protein components of plasma recognized by physico-chemical means has thus increased to nine or more from the three recognized a century ago

Hormones A very large number of protein components of the plasma has been postulated on the basis of physiologic or immunologic properties Some of these have been concentrated and characterized Others like the hormones by definition components of the blood have rarely been separated from it or even detected in it

Immunoproteins Immunologic studies have led to the recognition and the study of complement and its components[25-30] and of a variety of antibodies [31] Antibodies have been characterized as euglobulins and as pseudoglobulins in terms of their solubilities Many but not all antibodies have been characterized as γ globulins in terms of their electrophoretic mobilities Among γ globulins some have been described of very high molecular weight others of molecular weights of the order of 156 000 For the most part however these immuno chemical studies have not led to the isolation of pure antibodies of which there could conceivably be as many as the antigens which have led to the production by the body of specific antibodies

Enzymes Prothrombin is presumably present in the body in but small amount and the action of thrombin is now generally regarded as enzymatic The presence of a large number of other enzymes has since been demonstrated by virtue of their specific interactions Thus there is the proteolytic fibrinolytic enzyme now called plasmin a well defined serum esterase two phospharases a lipase an amylase and a number of peptidases among them hypertensinase Most of these substances are far better known in terms of their chemical interactions than of their molecular properties

Lipoproteins Recent investigations have demonstrated the presence of different lipoproteins in the plasma moving in the electric field respectively with the mobilities of α_1 and β_1 globulins One of these lipoproteins is an asymmetric molecule with a molecular weight of roughly 200 000 Another is a large spherical lipoprotein with a molecular weight of over a million [17] These lipoproteins are noteworthy both because of their physical properties and because they render soluble such water insoluble lipids as cholesterol carotene and the steroids and because the specificity of their interactions is such that one of the estrogen hormones estriol has been found to be combined not with all but only with one of these lipoproteins the large spherical β_1 lipoprotein[3]

Albumins Until recently the greatest emphasis has been upon the osmotic function of the albumins in maintaining the equilibrium between water and electrolytes in the blood and in the tissues The development of normal human serum albumin for use in military medicine for the treatment of shock burns and hypoproteinemia depended upon the molecular properties of albumin However as Bennhold[33] and later investigators[34] suggested albumins interact with a variety of smaller molecules notably with nonpolar anions such as aliphatic fatty acids[35–37] and are presumably responsible for their transport in the blood stream Albumins also interact with a variety of dyes[33] including Evan s blue often used in estimating blood volume[35] with naphthoquinones such as those developed as antimalarials[39] and with a variety of other dyes [40–4] Albumins also combine with a variety of drugs such as atabrin neosalvarsan [33] and digitoxin [43] mercurials[44] and sulfa drugs [45 46]

Crystallized Human Serum Albumins Not all of the properties that have been ascribed to the albumins are due to these molecules In order to demonstrate this it was necessary to prepare highly purified crystallized human serum albumins [47]

Human serum albumins that had been crystallized by earlier methods were demonstrated to contain over 2 per cent of long chain fatty acid [48] The albumins that we have crystallized in very satisfactory yield from alcohol water mixtures of defined pH and ionic strength at low temperatures also contained fatty acid but the amounts present were far smaller of the order of one mole stearic or oleic acid per mole of albumin The fatty acid appeared to form an integral part of the crystal structure and in fact crystallization appears to be greatly aided by the presence of such amounts of fatty acid and by the addition of higher alcohols such as n decanol The amounts of the alcohol that have been found useful and with which the albumins combine range from two to ten moles per mole Crystallized with the aid of such reagents albumins can be recrystallized under a variety of physical chemical conditions and in a variety of crystal forms

The resolution of the various albumins that crystallize together required a more specific method of crystallization in order to yield chemical individuals Conditions for crystallizing horse serum albumin of constant solubility had been determined in our laboratory before the war by McMeekin [49] This method has thus fa not been found effective for crystallizing a fraction of human serum albumin However a large fraction of the human serum albumins crystallized by the decanol method

has been found by W L Hughes Jr to form a relatively insoluble crystalline mercury compound [50] The albumin separated in this way appears to be a chemical individual whose solubility behavior approximates to that of a simple chemical substance

Although serum albumins combine with a larger number of equivalents of mercury the amount with the albumin in the solid phase of crystals precipitated in this way is but one half a mole of mercury per mole albumin That is to say each mole of mercury appears to be combined with two albumin molecules in the solid state [50] In solution however this complex dissociates Albumin of double molecular weight has been detected in the ultracentrifuge and reconverted to normal size either by the removal or the addition of larger amounts of mercury

Pigment Proteins Serum albumin had previously been reported to combine with hematin and with bilirubin [52] Upon adequate recrystallization the amount of both diminish until they can no longer be readily detected spectrophotometrically Upon equilibrating such pure serum albumin with these substances however combination can be demonstrated and quantitatively estimated Albumin which we have recrystallized has been studied in equilibrium with hematin[51] and with bilirubin [4] At alkaline reaction albumin combines with as much as three moles bilirubin per mole albumin At acid reactions however the bilirubin is free and can be removed by dialysis

Bilirubin is also a component of a true pigment protein of the blood stream normally present to less than a tenth of a per cent of the plasma proteins This bilirubin pigment protein interacts strongly and is therefore separated with difficulty from the 50 per cent of albumin in plasma It has now been separated however and alone of adequately purified plasma proteins give the indirect van den Bergh reaction [53] Another pigment protein responsible for a very characteristic blue green color does not give this reaction and although often found associated with crude albumin preparations is a globulin concentrated in our system of fractionation in Fraction IV 1 whereas the yellow pigment due to bilirubin is concentrated in Fraction V 1

Iodoproteins Iodine combines with essentially all proteins entering the phenol ring to form diiodotyrosine and also entering the imidazol ring Albumin rich in iodine has been prepared and crystallized by Salter from horse serum [54] In studying the distribution of iodine in the human plasma fractions that we have separated some has always been found with the albumin some however has been found in Fraction IV 6 The further study of the iodoprotein in these fractions should reveal more regarding the nature of the plasma molecules of which it is a part

Metal-Combining Proteins It has long been known that copper iron and zinc are combined by plasma protein The separation of the plasma proteins into fractions in which are concentrated the molecules responsible for specific interactions has yielded in Fraction IV 7 the β_1 globulin responsible for the combination and transport of copper and iron and perhaps of zinc in the plasma The close interrelation of the copper and iron in plasma had been noted in clinical studies [55–57] The combination of a component of plasma with iron was noted in connection with

TABLE 1 —*Protein Components of Human Plasma Separated and Concentrated in Diverse Fractions*

Protein Component	Estimated Amount in 100 g Plasma Protein	Concentrated in Fraction §	Approximate Isoelectric Point	Specific Chemical Interaction
	grams			
Fibrinogen	4	I 2	5 3	Thrombin
Non clottable protein insoluble at low temperature	0 15	I 1		
Antihemophilic globulin		I		
Antibody γ—globulins	11			
Diphtheria antibodies*	(0 001)			
Measles antibodies				
Mumps antibodies				
Streptococci antitoxin*		II	7 3	Antigens
Influenza antibodies				
Pertussis antibodies*				
Typhoid H agglutinins*				
Antibody euglobulins		III 1	6 3	Antigens
Typhoid O agglutinins				
Isoagglutinins	(0 03)	III 1	6 3	Incompatible Red Blood cells
Anti A anti B				
Anti Rh antibodies				
Complement components				
C 1	0 4	III 2†		Antigen antibody complex
C 2		IV †		
Enzyme precursors				
Prothrombin	0 3	III 2		Thromboplastin
Plasminogen		III 3		*Streptokinase*
Serum enzymes				
Thrombin		III 2	4 8	Fibrinogen
Plasmin		III 3		Proteins
Amylase				Starch
Lipase				Lipid
Peptidase		IV		l Leucylglycylglycine
Phosphatase (alkaline)		IV †		Phosphoric acid monoesters
Esterase	0 02	IV 6	4 5	Acetylcholine ethylbutyrate
Metal combining β —Pseudoglobulin crystallized	2 5	IV 7	5 6	Iron and copper
High molecular weight β_1*—globulins* (lipid poor)				
S= 7	2	III 0		
S=20	1	*III-0*		
Iodoprotein*‡		IV 6		

TABLE 1—Continued

Protein Component	Estimated Amount in 100 g Plasma Protein	Concentrated in Fraction§	Approximate Isoelectric Point	Specific Chemical Interaction
	gm			
Thyrotropic hormone		IV-4		
Glycoproteins				
α_2 Glyco pseudoglobulin	0.7	IV-6	4.9	
α_2 Mucoid globulin	0.5	IV-6	4.9	
Lipoproteins				
β_1 75% lipid containing X protein	5	III-0	5.6	Estriol carotenoids and other steroids
α_1 35% lipid-containing protein	3	IV-0	5.2	Steroids
Blue green pigment α globulin		IV-2		
Bilirubin containing α_1 globulin‡	0.03	V-1	4.7	Diazo reaction
Albumin crystallized with mercury		V	4.9	Mercury decanol
Albumin crystallized with decanol	30	V	4.9	Fatty acids bile salts many dyes and drugs

* These components represent but small proportions of the fraction and subfraction and their properties cannot therefore be deduced from those of the concentrates in which they have been separated

† These components have not been tested for since revision of the fractionation process

‡ Albumin binds more bilirubin than the bilirubin pigment globulin in Fraction V 1 and more iodine than has been found in Fraction IV 6

§ When purified chemical components have been separated from fraction they have not been given new fraction numbers In that case the fraction number refers to the starting material for the separation of the component

bacterial studies[55] and has led to its identification in a plasma fraction to its further purification and characterization and recently to its crystallization in collaboration with Bernhard A Koechlin[59-61] in our laboratory Physiologic studies of the role of the separated protein injected into man have begun[6, 54] with a view to determining its function and possible value in therapy

Specificity of Chemical Interactions The multivariable method for the fractionation of plasma that we have developed not only separates protein components in terms of their molecular properties but also in terms of the very specific chemical configurations upon which their interactions depend Insofar as the system of fractionation is successful each protein component responsible for a specific chemical reaction and physiological function is separated from those of different properties and concentrated Thus one fraction should be positive with respect to any test to which the whole plasma is positive all others should be negative The extent

to which this end has been accomplished may be demonstrated by the following studies upon protein interactions *

1 Interaction of Naphthaquinone (M1523†) Caprylate and Serum Albumin

Solutions

Five per cent solutions of the following proteins are used Volume of each solution 300 cc
γ Globulin (Fraction II) in acetate buffer pH‡ 5 7 Γ/2 = 0 05
β_1 Globulin (Fraction IV 7) =
Albumin (Fraction V) =
Albumin (Fraction V) in caprylate =
An acetate buffer blank =
Albumin control in acetate buffer as above but with naphthoquinone added about 6–12 hours in advance of the demonstration time

Reagent

Naphthaquinone M1523§

Method of Demonstration

To beakers containing each of the above solutions a small amount of finely reprecipitated naphthoquinone is added stirred and let stand 10–20 minutes A beautiful red color develops in the albumin solution containing acetate but is blocked in the albumin solution containing caprylate The other solutions and blank give fainter colors

2 Binding of Bile Salts by Serum Albumin‖

Solutions

Serum Albumin 25 per cent solution

Reagents

Bile Salts 2 per cent solution
Red cell as an indicator
0 15 M sodium chloride solution

Method of Demonstration

Two beakers each containing a suspension of 3 cc packed human red cell in 600 cc 0 15 M NaCl solution are prepared in advance To one beaker 60 cc of a 25 per cent solution of serum albumin is added with stirring Next 45 cc of a 2 per cent solution of bile salts is added to each beaker stirred and let stand

In 10–15 minutes the suspension of cells containing bile salts only is hemolized the other containing albumin is essentially unchanged

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These demonstrations have been prepared by L H Larsen in collaboration with various members of this Department especially L E Strong and in the case of Demonstration I H A Saroff II W L Hughes Jr III B A Koechlin IV N H Martin and B J Livingstone and V J D Ferry and P R Morrison

† 2 hydroxy 3 isopentyl x naphthoquinone an antimalarial obtained from L F Fieser

‡ If the pH is higher all solutions and buffer blank will give a reaction but color in the one albumin solution will always be most intense

§ The reaction is more rapid if the naphthoquinone is freshly prepared as a paste by dissolving in alcohol and reprecipitating with water It must be washed alcohol free otherwise it will give a reaction when added to any of the solutions

‖ This experiment was suggested by B D Davis and R J Dubos[65]

3 Bind ng of C pp and I on by β-Global n (F act on II)

S t n

2 per cent soluti ns of the foll wing prote ns are used Volume of each solution 300 cc
Fibrinogen (Fraction I)
γ-Gl bulin (Fraction II)
β_1-Gl bulin (Fraction IV) } All are di solved in pH 8 5 Γ/2 = 0 05 barbiturate buffer
Albumin (Fraction V)
β Gl bulin (Fracti n IV) *control* }

Reagents

1 $CuSO_4$ 5 H_2O
Four bottles each conta ning 0.02 Gm $CuSO$ 5 H_2O di solved in 20 cc H_2O
2 FeSO (NH)SO 6H O
F ur bottles each containing 0.045 Gm FeSO (NH)SO 6 H O dissolved in 20 cc 0.001 M acetic acid
3 One bottle conta ning 27 cc 1 M acetic acid
4 One bottle containing 27 cc 1 M NaOH

M thod f Demonst at on

All solutions must be stirred during addition of re gents
1 *C pp B d ng*
Add copper solution to all protein solutions β-Globulin is the only protein solution that will give a po itive re ction A yellow green color develops in 3 5 minutes pH is about 8 8.2
2 *I on B nding*
Add iron solution to all protein solutions β_1-Globulin again gi es the only positive reaction A red color devel ps in 3–5 minutes as the copper is replaced by the iron pH i about 8 8 2
3 *Split g Iron the P t in‡*
Add the acetic acid The protein soluti n regains its original appearance pH is about 4 3
4 *R comb n ng th I on*
Add the sodium hydroxide The red color reappears pH is about 8 8 2
N t The yellow green color of the copper and the red color of the iron become some what more intense with time

4 Mod fi d an d n Bergh R a tion for D nt fi cat n f B l ub n

S lut ns

2 per cent olutions of the following pla ma f actions are used Volume of each 120 cc
Fraction II
Fraction IV 1
Fraction IV } *All solutions are made up in pH = 8 6 Γ/2 = 0 0 5* §ba biturate buffer
Albumin
Fraction V 1 B

The eagent listed below a e added to the control about 4–6 hours in ad ance of the d monstration time

† This pH is lowered slightly upon the add tion of the copper and iron salts due to cidity of the alts themsel e and because 0 00 M acetic acid is used to d ssolve the FeSO (NH)SO_4 6 H O

‡ It s nece ry only to add c d and b se to the β Globul n solut on ince it alone gives a po itive re ction to the iron

§ The ion c strength of F action II s adjusted to 0 04 with 1 M NaCl to keep it from precipitating on addition of re gents If h gher ion c t ength for IV 1 and V 1 B is used they ill precipitate on adding reagent

Reagents

1 Solution A

1 g Sulfanilic acid dissolved in 15 cc concentrated HCl and diluted to 1 liter with water

2 Solution B

0 5 per cent $NaNO_2$

3 Diazo reagent prepared fresh by adding solution B to solution A in the following ratio 0 3 cc B to 10 cc A

4 Absolute methanol

The reaction mixture to be added to the protein solutions is prepared about an hour before the demonstration as follows

Add 5 7 cc solution B to 190 cc solution A Add 190 cc of this diazo reagent to 950 cc absolute methanol *

Method of Demonstration

Add 180 cc of the above reaction mixture to each of the protein solutions with vigorous stirring A precipitate may form but will redissolve

The V 1 B solution will develop a pink color almost instantaneously The other solutions give a negative reaction

5 *Coarse and Fine Types of Fibrin Clots*†

Solutions

A 2 per cent solution of Fraction I is prepared for this demonstration

Note The Fraction I used is dried from the frozen state from a 2 per cent isotonic solution in sodium citrate volume 300 cc and it is only necessary to add 300 cc distilled water to reconstitute it pH = about 6 3 6 6

After the Fraction I is dissolved it is clarified by filtering successively through D-0 and D 5 Horman filter pads These pads may be first washed with 0 1 M acetic acid then with water to free them of acid

After filtration the solution is divided into two 125 cc portions One remains as it is at pH 6 3 6 6 the other is adjusted to about pH 8 by the addition of 18 8 cc 1 M $NaHCO_3$ solution

Reagent

The only reagent necessary is a thrombin solution made by dissolving two small bottles of thrombin (about 500 units each) in 50 cc 0 15 M NaCl solution

Note If this solution is cloudy it is clarified by filtration through a small D 5 Horman filter pad

Method of Demonstration

Coarse Clot To the pH 6 3 portion of Fraction I 12 5 cc thrombin solution are added and stirred quickly to mix the two solutions

In about 5 minutes a clot forms firmly enough to permit inversion of the container This clot is opaque

Fine Clot To the pH 8 portion 12 5 cc thrombin solution are added and stirred quickly

In about 5 minutes or slightly longer a firm clot forms so that the container can be inverted This clot is clear *N t* This clot forms a little more slowly than the coarse clot

SUMMARY

The chemical methods that have been developed for the separation concentration and purification of the protein glycoprotein mucoprotein and lipoprotein

* Reaction mixture should not develop a color If a color develops it is probably due to an impurity in one of the reagents Therefore fresh reagents should be prepared

† This demonstration depends upon the experiment of J D Ferry and P R Morrison [68]

components of any biologic system by fractionation in alcohol water mixtures at controlled pH salt and protein concentration at the subzero temperatures necessary to prevent denaturation have thus far led to the recognition and concentration of over twenty five different protein components of human plasma These include albumins of more than one kind immune globulins which differ in their physical properties and interactions with antigens lipoproteins which differ in their physical properties and interactions with steroids enzymes with protease peptidase lipase phosphatase and esterase activity thrombin fibrinogen and the antihemophilic globulin concerned with blood coagulation iodoprotein and the recently crystallized metal-combining protein which interacts with both copper and iron and is presumably concerned with transport in the plasma

This number of plasma proteins is far greater than can be detected electrophoretically or in the ultracentrifuge Chemical fractionation has yielded at least four β_1 globulins and at least two α_1 and three α_2 globulins The α_2 globulins include a mucoprotein and glycoproteins of more than one kind the α_1 globulins the bilirubin containing globulin in Fraction V 1 and the lipoprotein in Fraction IV 1 The β_1 globulins include the carotene rich euglobulin which combines with three times its weight of lipid as well as a high molecular weight lipid free β_1 globulin both of which are concentrated in Fraction III-0 Fraction III also contains β_1 globulins of different molecular properties The iron binding component of the plasma crystallized from fraction IV 7 is a lipid free β_1 globulin and is more closely related to the albumins than to other globulins from the point of view of osmotic activity Electrophoretically indistinguishable these different β_1 globulins have no other common property The lipid binding plasma component is a β_1 euglobulin the iron binding β_1-component a pseudoglobulin They differ in size shape in solubility in chemical composition and interaction and in physiological function

The separation and concentration of the various proteins of human plasma was undertaken during this war in order to render as many as possible available as therapeutic agents and thus to increase our knowledge and control of the composition of the blood in health and in disease Many more have been separated and are being studied chemically than have thus far been brought to clinical trial Their study renders possible the further investigation of the chemical specificity of the interactions of the plasma proteins which are responsible for many of the tests that have in the past or may in the future prove of value in the clinic in the study of pathological sera and the understanding of the specific protein component that is either elevated or deficient in this condition

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STUDIES IN IRON TRANSPORTATION AND METABOLISM

VI Absorption of Radioactive Iron in Patients with Fever and with Anemias of Varied Etiology*

By Reubenia Dubach Ph D Sheila T E Callender M D and Carl V Moore M D

RECENT studies of iron utilization have demonstrated that intravenously administered tracer doses of radioactive iron are utilized completely for hemoglobin synthesis by afebrile iron-deficient patients or animals and almost completely by normal subjects [1] On the other hand when hemoglobin synthesis is impaired as in patients with refractory anemia or untreated pernicious anemia in patients with febrile disorders and in pigs with infection or pyridoxine deficiency both the rate and completeness of utilization are decreased [1-3] It was discovered furthermore that the amount of tagged iron which appears as hemoglobin in the peripheral blood of subjects with hemolytic anemia cannot be used as a measure of iron utilization because of the rapid rate at which isotopic hemoglobin is removed from the circulation These results clearly indicate that the radioactive iron technic for studying iron absorption as used in the past [4-12] may give erroneously low values because only iron built into hemoglobin is measured the assumption that this amount equals the quantity absorbed is not always justified In the face of this new evidence it is necessary to re-evaluate the ability of patients with impaired hemoglobin formation to absorb iron Absorption of the metal by normal subjects should also be restudied because even though normal persons use tracer amounts of injected iron completely or almost completely for hemoglobin synthesis absorbed iron goes into the portal rather than the systemic circulation and may therefore be handled in a different way This report describes experiments designed to meet the above objections and to discover the physiologic pattern of iron absorption under a variety of pathologic influences

The isotopic method for studying iron absorption has been extended to include not only measurement of the amount of iron converted into hemoglobin after an oral dose but also determination of the unabsorbed portion which is eliminated in the feces A standard dose of 1 mg of iron per kilogram of body weight has been selected Any portion of the test dose not accounted for in the circulating hemoglobin and in the recovery from feces represents iron that has been absorbed but not immediately utilized With this approach the principle that iron deficient subjects absorb larger amounts than do normal persons has been confirmed [4, 13, 14] The amount retained by healthy men and women however has occasionally exceeded 10 per cent because of the error inherent in the method it was not possible to determine accurately how much might be stored without being used immediately for hemoglobin but the quantity was not large It was possible to demonstrate that patients with refractory anemia untreated pernicious anemia

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and fever often did absorb more iron than they were able to build into hemoglobin during the period of observation One woman with Hodgkin s disease and hypo chromic anemia for instance retained 50 per cent of the oral dose but used for hemoglobin synthesis only one third of the amount absorbed

These results are of considerable theoretic interest It is now believed that the animal organism has an extremely limited capacity to excrete iron[14, 15] except by hemorrhage and that the intestinal mucosa protects the body from accumulating toxic amounts of the metal by accepting or rejecting iron according to need [8, 16, 17] The intestinal mucosa according to this concept is one of the major regulators of iron metabolism Evidence indicating that patients with hypochromic anemia absorb more iron than do normal persons is compatible with this theory However the observation that patients with untreated pernicious anemia and refractory anemia absorb appreciable amounts of the metal in spite of the relatively large amounts of iron in their tissues shows the regulatory effect of the intestinal mucosa to be less precise than was formerly believed

Material and Methods

The subject who volunteered for this study were healthy medical students members of the laboratory staff and patients on the Medical Service of the Barnes Hospital The test dose of radioactive iron was given orally at a level of 1 mg of iron per kilogram of body weight as ferrous chloride reduced from the ferric state with ascorbic acid The usual procedure was to give the test dose after a night s fast but in a number of experiments the iron was given 3 to 5 hours after a meal In one instance through an error the test dose was given immediately after the patient had eaten lunch and excellent absorption of the metal occurred (J L first dose table 2 fig 1) Quantitative fecal collections were made until radio activity measurements showed that the 24 hour specimen contained less than 0 5 per cent of the activity in the test dose In most of the subjects fecal collections could be suspended after the sixth or seventh day In two instance they were continued until the twelfth day Since the method depended on getting complete stool collections extreme vigilance was exercised by the laboratory staff Only experiments in which there was satisfactory evidence of reliable collections have been included in the study Blood was drawn at intervals of three days for determination of the radioactive iron in hemoglobin The total amount of radioactivity in the p ripheral blood was calculated by assuming the blood volume to be 80 cc per kilogram of body weight The error introduced by this assumption did not influence inter pretation of results since the amount of iron found in the blood as usually small as compared with that recovered from the feces Details of the technics for making the radioactivity determinations have already been published [13]

The fresh fecal specimen was weighed and mixed thoroughly with water to which enough concen trated hydrochloric acid was added to bring the final concentration to approximately 0 6 N (50 cc concentrated HCl per liter) The su pension was transferred quantitatively through a funnel to a liter volumetric flask and eno gh wat r was added to adjust the volume to exactly one liter The flask was stoppered and shaken igorou ly f r bo t three minutes As quickly as pos ible an aliquot sample (one twentieth to one tenth) of the sto l su pension was measured into a Kjeldahl flask The mixture was then digested with sulfuric and pe chlo ic cids The cooled digest was transferred to a volumetric flask and an aliquot portion as taken f me surement of radioactivity Five milligrams of inert iron were added as a carrier to the aliquot n a 40 cc cent fuge tube and the iron as precipitated with NaOH using phenol red as an indicato The p ec p t te as th own down by centrifugation the supernatant liquid was discarded and the precipit te s di sol ed in 0 3 cc of 3 M H SO To this solution were added in the rentrif ge tube about o c of a mixture of three parts of saturated ammonium oxalate solution and one part of saturated oxalic acid solution The precipitated calcium and magnesium oxalate salts were thro vn down by centrifugation and the supern t nt ol tion w s tran ferred qu ntitatively to the electroplating cell The prec pitate of oxalates as tirred with a few drop of 3 M H SO and 10 cc of the oxalate mixture the tube was again centrifuged and th washings were combined ith the

material already in the electroplating cell From this solution the iron was electroplated onto a copper disk[13] and its radioactivity was measured with a bell type Geiger counter tube

Attention is directed to the fact that iron was not extracted from the fecal specimens by ether as was done in certain earlier experiments [3] Ether extraction was unnecessary because the radioactivity in these fecal specimens was great enough to permit high dilutions of the specimens In these high dilutions salts other than calcium and magnesium did not interfere with the determination

TABLE 1 —*Efficiency of Recovery of Radioactive Iron Added to Feces*

Sample	Weight of Fresh Feces	Chemical Form of Radioiron Added	Method	Aliquot Counted	Radioiron Added	Radioiron Found	Recovery
	Gm				counts per min	counts per min	%
1	66	$FeCl_3$	Ether extraction	1/6 5	64	593	93 5
2	[illegible]	$FeCl_3$	Oxalate precipitation	1/50	64 320	64 320	100
3	[illegible]	$FeCl_3$	Oxalate precipitation	1/100	125 600	131 600	10[illegible]
4	[illegible]	$FeCl_3$	Oxalate precipitation	1/100	25 200	233 [illegible]	91
5	50	$Fe(OH)_3$	Oxalate precipitation	1/5000	3 600 000	3 396 000	94
6	50	$Fe(OH)_3$	Oxalate precipitation	1/5000	3 600 000	3 490 000	9[illegible]
	50	$Fe(OH)_3$	Oxalate precipitation	1/2500	1 440 000	1 352 000	94
8	50	$Fe(OH)_3$	Oxalate precipitation	1/1000	[illegible] 000	668 000	93
9	50	$FePO$	Oxalate precipitation	1/1000	1 440 000	1 431 000	100
10	50	$FePO$	Oxalate precipitation	1/2000	1 440 000	1 374 400	95 5
11	40	$FePO$	Oxalate precipitation	1/20	29 500	[illegible]	91
12	40	$FePO_4$	Oxalate precipitation	1/100	9 500	98 200	101

TABLE 2 —*The Absorption of Radioactive Iron by Patients with Hypochromic Anemia*

Patient	Date	Hematologic Data: R.B.C.	Hb	C.V.	MCV	MCHC	Total Test Dose of Radioiron		Radioiron Recovered: Feces	Blood	Total
		m	Gm	%	cu micron	%	mg/kg	counts per min	%	%	%
J L	1-24 45	4 24	9 1	32	6	28	1	4 440 000	38	57	95
J L	3 26 45 †	4 23	8 5	30	1	28	1	3 360 000	6	24	100
O J	10-7 46	3 81	5 4	2	58	24	1	1 280 000	64 5	36	100 5
M M	3 12-4	3 6	1	2	2	26	1	1 9 0 000	50	5	10
H N	5-3 4	4 93	4	28	5	26	1	1 850 000	60	27	87
S W	8-11-4 †	4 06	6 9	24	39	24	1	32 000 000	9	81	110

Forty eight hours before the second test dose of radioiron J L received 1310 mg Fe as colloidal Fe(OH intravenously

† The blood volume of J L and of S W was measured by the Evans blue dye method

H N vomited four hours after taking the test dose of radioiron

Prior to October 1946 the radioactive isotope used was Fe^{59} prepared in the Washington University cyclotron Since that time we have used a mixture of Fe^{55} and Fe^{59} obtained from the Clinton Laboratory On each day that determinations of radioactivity were made the number of counts emitted by a standard prepared from the original iron solution was also determined several times this value was used as the standard of reference for all calculation

RESULTS

1 THE ACCURACY OF THE METHOD

Twelve recovery experiments were done to test the accuracy of the determination of radioactive iron in feces (table 1) In the first four measured amounts of radio-

iron as ferric chloride were added to four different fecal suspensions which had been acidified with HCl Because radioiron of weak activity was added to a large mass of feces in the first experiment ether extraction according to the method previously described[1] was used ether extraction was not done during any of the remaining determinations described in this report In the next six experiments the isotope in the form of ferric hydroxide ferrous hydroxide or ferric phosphate was added to the fecal specimen carefully mixed and incubated at 37°C for twenty four hours before the determination was made in the last two experiments radioactive iron as ferric phosphate was added to the solid specimen and an excess

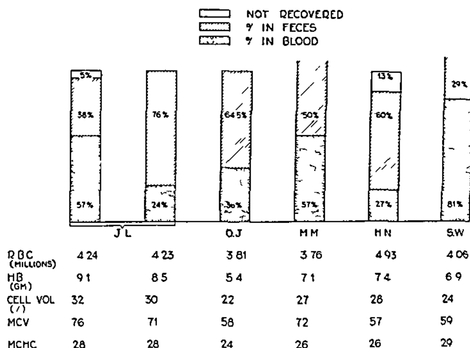


Fig 1 Recovery of an Oral Dose of Radioiron in Hypochromic Anemia
Dose 1 mg Fe per kg as FeCl

of N NaOH was added Recovery varied from 91 to 102 per cent the form in which iron was added did not influence recovery

Since iron deficient subjects promptly and quantitatively utilize any iron available to them for hemoglobin synthesis [1] it was thought that a study of iron absorption in such a group would provide a further means of checking the accuracy of the method In these patients if the method is valid the sum of the radioiron found in the circulating blood and that recovered from the feces should equal the amount given orally in the test dose Six experiments on patients with hypochromic microcytic anemia were done (table 2 fig 1) In only one was the recovery less than 95 per cent this patient vomited four hours after taking the test dose and failed to save the vomitus It is possible that a small amount of the radioiron was lost in this way In five experiments from 95 to 110 per cent of the ingested isotope was recovered in the blood and feces

These two types of observations define the accuracy of the method The error in

recovering iron from feces may be as great as 10 per cent The sum of the amount found in circulating hemoglobin plus that recovered from the intestinal tract should not be in error by an amount greater than plus or minus 12 per cent

NORMAL SUBJECTS

Absorption of radioiron was measured ten times in eight different normal subjects (table 3 fig 2) The amount accounted for in blood and feces varied 83-100 per cent of the administered dose in only two instances was the value less than 90 per cent Comparison of the amount of radioiron retained by the body (activity in the test dose minus that recovered in the feces) with the amount found in circulating hemoglobin indicates that in 9 of the 10 determinations some iron was

TABLE 3 —*The Absorption of Radioactive Iron by Normal Subjects*

Subject	Date	Hematologic Data					Total Test Dose of Radioiron		Radioiron Recovered		
		RBC	Hb	C H V I	MCV	MCHC			Feces	Blood	Total
		mill	Gm	%	cu μ	%	mg Fe	[illegible]	%	%	%
R D	4 10-46	4 63	14 2	43	93	33	1	600 000	91	7	98
S C	1 6 47	4 10	14 2	42	102	34	1	3 280 000	86	6	92
S C	3- 5-4	4 08	14 4	40	98	36	1	1 630 000	87	10	97
J T	9 16 46	4 82	15 0	44	91	34	1	2 700 000	98	2	100
J T	4- 8 47†	4 84	16 3	45	93	36	1	2 540 000	79	4	83
T K	2 13 47	5 32	17 9	46	87	39	1	3 040 000	83	7	90
C M	1 28 4 *	3 64	16 8	46	82	37	2	3 360 000	88	9	97
G G	2 8 47§	4 80	16 4	47	98	35	1	2 400 000	82	9	91
E F	2 20-4	4 86	16 1	47	97	34	1	2 090 000	81	7	88
J N	8- 9 47‖	4 52	14 2	44	97	32	1	43 520 000	79	11	90

Cohn s protein IV 7 (4 5 Gm protein) was given i v immediately after the radioiron
† Cohn s protein IV 7 (24 Gm protein) was given i v immediately after the radioiron
Cohn s protein IV 7 (3 75 Gm protein) had been given i v twenty four hours before the radioiron
§ G G vomited 6 hours after taking the radioiron
‖ The blood volume was determined by the Evans blue dye method

absorbed but not utilized Even though this quantity amounted to 17 per cent in the second experiment on J T and was 10 per cent or more in three other instances it was never large Because of the error of the method it can only be stated that the results suggest that normal persons absorb more iron than they build into hemoglobin under these conditions

There are however certain conclusions which can be made with assurance Patients with hypochromic anemia *do* absorb several times more iron from test doses of this magnitude than do normal persons On the other hand normal subjects retain greater amounts than Hahn and his associates originally indicated might be the case [4] Even though the intestinal mucosa may be one of the principal regulators of iron metabolism as has been suggested it is not so efficient a regulator that it causes normal subjects to reject iron almost completely

In three of the experiments listed in table 3 a quantity of the iron binding globulin of plasma Cohn s fraction IV 7 * was given intravenously either before or after the oral test dose of iron This was done to see whether absorption would be increased if considerable quantities of this globulin were circulating during the period of absorption The differences were not great enough to justify any conclusion if there was an increased absorption the increase was certainly small

3 PATIENTS WITH ANEMIAS OF VARIED ETIOLOGY OR FEVER

Iron absorption has been studied in 12 patients selected because they had diseases in which utilization of absorbed radioiron might well have been incomplete

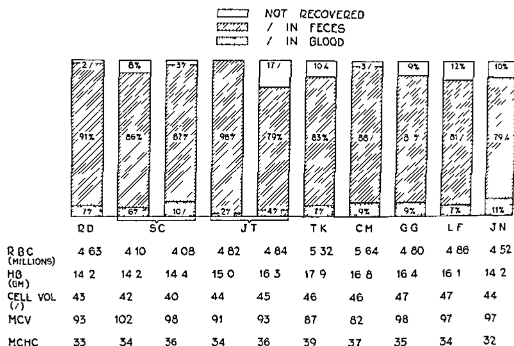


Fig 2 Recovery of an Oral Dose of Radioiron Given to Normal Subjects
Do e 1 mg Fe per kg a FeCl

(table 4 figs 3 4) We wished to know (1) how well these persons absorbed iron and (2) how erroneous the results would have been if the amount of radioiron found in circulating hemoglobin had been taken as the sole measure of absorption Because of the error in recovering iron from feces no conclusions can be drawn from the results on patients 5 10 and 12 even though the amount of radioiron found in their peripheral blood was negligible For similar reasons caution is necessary in interpreting the results on patient 6 Patient 12 might have shown greater absorption had she not had unusually rapid intestinal action Over 60 per cent of the iron given appeared in a stool passed within eighteen hours of the dose In the remaining observations however there is no question about the fact

* Obtained through the courtesy of Dr E J Cohn

that more iron was absorbed than could be accounted for in the hemoglobin of circulating blood Experiments 8 and 9 demonstrate this particularly well These two patients had Hodgkin s disease associated with fever and hypochromic anemia

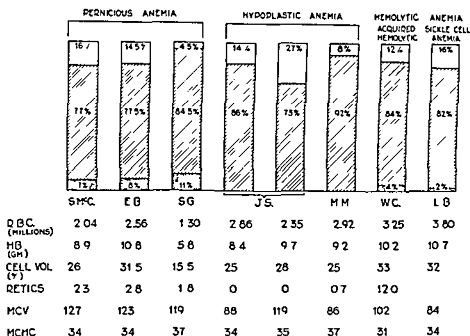
Table 4—Th Ab orpti n f Rad act e I n by Pat ents w h I ane as B e d Dy rasias or Inf c n

I t t	Diagn	D t	RBC	Hb	C I	Ret	MCV	MCHC	T t IT t Do (Ra) n		Radioir Rec red		
			ml	Gm					mg/kg				
1 S McC	Addisonian pernicious anemia	12-6 4	2 04	8 9	26	2 3	12	54	1	3 320 000		~	84
2 E B	Addisonian pernicious anemia	1 11 47	2 56	10 8	31 5	2 8	1 3	34	1	3 232 000	~ 5	8	85 5
3 S G	Addisonian pernicious anemia	6-6-4	1 30	5 8	15 5	1 8	119	3	1	4 800 000	84 5	11	95
4 J S	Refractory anemia	10-1-46	1 86	8 4	5	1 0	88	34	1	2 220 000	86	0	86
J S	Refractory anemia	2 18 4	2 35	9	28	0	119	33	2	4 050 000	3	0	3
5 M M	Refractory anemia	4 21 4	2 9	9	2 25	0	86	37	1	1 600 000	92		0 2 9
6 W C	Acquired hemolytic anemia	10-3 46	3 25	10 2	33	12 0	102	31	1	1 460 000	84	4	88
L B	Sickle cell anemia	8 21-4	3 80	10	3		84	34	1	4 800 000	82	2	84
8 D G	Hodgkin disease	12 13-46	4 34	9 3	32	2	74	29	1	3 950 000	48	15	63
9 J W	Hodgkin s disease	4 2-4	3 41	5 4	22	2 8	65	24	1	1 450 000	68	2 5	70 5
10 S D	Diabetic gangrene	4 28 4	5 29	17	1 50		95	34	1	2 000 000	94	0	95
11 J C	Hemochromatosis	6-10-47	4 83	16 3	49		101	33	1	5 200 000	78 5	2	80 5
2 C V	Leukemia	8 20 47	1 90	5 8	17		90	34		4 496 000	89 5	1	90 5

Secondary hemosiderosis from many transfusions

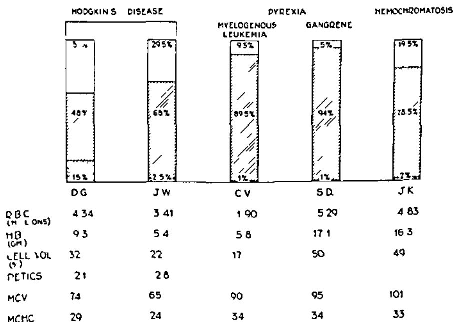
On the basis of fecal recovery they retained 52 and 32 per cent of the test dose yet utilized only 15 and 2 5 per cent respectively to build hemoglobin

Particular attention should be directed to the three observations on patients with pernicious anemia The data for one of these experiments are graphically illustrated in figure 5 Shortly after administration of the test dose of radioiron specific therapy in the form of liver extract was given The radioactive isotope



	SMC	EB	SG	JS		MM	WC	LB
RBC (MILLIONS)	2 04	2.56	1 30	2 86	2 35	2.92	3 25	3 80
HB (GM)	8 9	10 8	5 8	8 4	9 7	9 2	10 2	10 7
CELL VOL (%)	26	31 5	15 5	25	28	25	33	32
RETICS	2 3	2 8	1 8	0	0	0 7	12 0	
MCV	127	123	119	88	119	86	102	84
MCHC	34	34	37	34	35	37	31	34

Fig 3 Recovery of an Oral Dose of Radioiron in Anemias of Varied Etiology
Dose 1 mg Fe per kg as $FeCl_3$



	DG	JW	CV	SD	JK
RBC (MILLIONS)	4 34	3 41	1 90	5 29	4 83
HB (GM)	9 3	5 4	5 8	17 1	16 3
CELL VOL (%)	32	22	17	50	49
RETICS	21	2 8			
MCV	74	65	90	95	101
MCHC	29	24	34	34	33

Fig 4 Recovery of an Oral Dose of Radioiron in Pyrexia and in Hemochromatosis
Dose 1 mg Fe per kg as $FeCl_3$

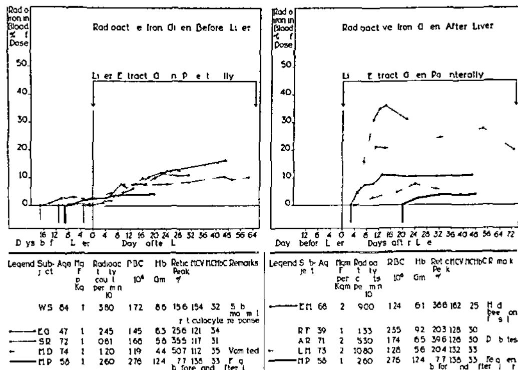
SG ♂ 58 yrs of age Addisonian Pernicious Anemia
Histamine Refractory Achlorhydria

Fig 5 Absorption of Radioactive Iron by a Patient with Pernicious Anemia

EG ♂ 47 yrs of age

Fig 6 Absorption of Radioactive Iron by a Patient with Pernicious Anemia

appeared slowly in this man s blood during a period of several months as he recovered from his anemia If the observations had been stopped at ten days, it would have been concluded from measurement of isotopic hemoglobin that only 2 per cent of the test dose had been absorbed yet 11 per cent eventually appeared in the blood An even more dramatic result is illustrated in figure 6 data for this experiment are not included in table 4 because fecal recovery was not obtained This patient had enough iron stored in his tissues to raise his hemoglobin from 7 to more than 12 grams per 100 cc after specific therapy was begun yet he absorbed more than 20 per cent of a test dose of radioiron This fact was completely masked



Legend	Sub- j ct	Age	Mg F p Kg	Radioac t ty cou t per m n 10	RBC 10^6	Hb Gm	Retic Peak %	MCV	MCHbC	Remarks
	WS	64	1	380	172	86	156	154	32	5 b ma m l r t culocyte re ponse
———	EG	47	1	245	145	63	256	121	34	
———	SR	72	1	081	168	58	355	117	31	
-	MD	74	1	120	119	44	507	112	35	Vom ted
———	MP	58	1	260	276	124	77	138	33	F q b fore and fter l

Legend	S b- je t	Age	Mgm F per Kgm	Rad oa t ty c ts pe m n 10	RBC 10^6	Hb Gm	Ret c Pe k %	MCV	MCHbC	R mark
———	EM	68	2	900	124	61	368	162	25	H d bee on f s l
	RF	39	1	133	235	92	203	128	30	
	AR	71	2	530	174	65	396	126	30	D b tes
-	LM	73	2	1080	128	56	204	132	33	
———	MP	58	1	260	276	124	77	138	33	Fe q en b for nd fter l r

Fig 7 Absorption of Radioactive Iron by Patients with Pernicious Anemia

during the period of relapse and became evident only as he recovered from his anemia Additional observations of a similar nature are recorded in figure 7 When the radioiron was given after therapy with liver extract absorption was occasionally greater than during the pretherapy period

Of particular interest also is the retention of over 20 per cent of the test dose by a patient with hemochromatosis while only 2 per cent appeared in his blood Even if the fecal recovery was low by 10 per cent he still absorbed five times as much as he built into hemoglobin This man had diabetes hepatomegaly and bronzing of his skin He had never received any transfusions to account for secondary hemosiderosis The diagnosis was confirmed by biopsy of both liver and skin

4 EFFECT OF LARGE DOSE OF INERT IRON GIVEN INTRAVENOUSLY ON ABSORPTION

To one of the patients with hypochromic anemia (J L table 2) 1345 mg of inert iron as colloidal ferric hydroxide were given intravenously This amount was

sufficient to increase his hemoglobin level from about 9 to over 12 grams per 100 cc Twenty one days prior to this injection the patient absorbed 57 per cent of a standard test dose of radioiron (figure 8) Two days after the intravenous therapy before he had converted much of the ferric hydroxide to hemoglobin and at a time when his tissues contained more than a gram of iron he absorbed 24 per cent of a second test dose Retention was less therefore after his tissues were well supplied with iron but was still relatively large

A similar result was obtained in another patient with hypochromic anemia these data are not listed in table 2 because fecal recoveries were not done This woman

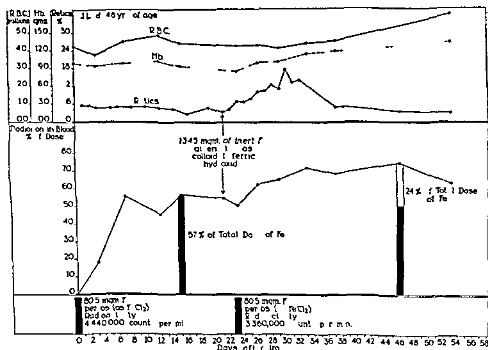
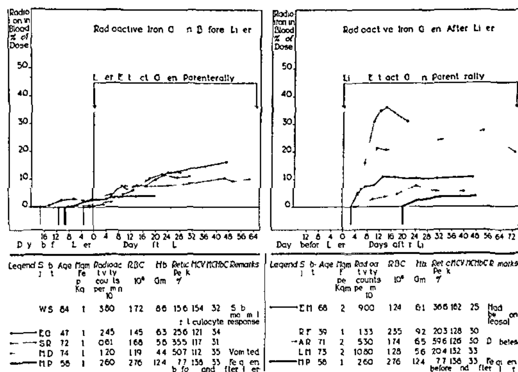


FIG 8 The Effect of a Large Dose of Parenterally Administered Iron on Iron Absorption

(A D) with a hemoglobin value of 9 4 Gm and a mean corpuscular hemoglobin concentration of 29 per cent absorbed 60 per cent of a test dose of radioiron in a control experiment Six weeks later she was given intravenously 755 mg of iron as colloidal ferric hydroxide On the eleventh day after this procedure when her hemoglobin had risen to 11 3 Gm and the mean corpuscular hemoglobin concentration was 30 per cent she was given a second dose of radioiron Of this 27 per cent appeared in her blood as hemoglobin Likewise when 480 mg of iron as colloidal ferric hydroxide were injected into a dog with depleted iron stores absorption was decreased from 14 9 per cent (control period) to 6 9 per cent These results are at variance with those of Hahn and his associates[8] who reported in one experiment that colloidal iron (304 mg) given by vein to an anemic dog did not significantly modify iron absorption

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Legend	Subject	Age	Mgm Fe per Kg	Radioactivity counts per min 10	RBC 10^6	Hb Gm	Retic Peak %	MCV	MCHbC	Remarks
	WS	64	1	380	172	86	156	154	32	Submaximal reticulocyte response
———	EG	47	1	245	145	63	256	121	34	
— — —	SR	72	1	061	168	56	355	117	31	
-	MD	74	1	120	119	44	507	112	35	Vomited
———	MP	58	1	260	276	124	77	138	33	Fe given before and after liver

Legend	Subject	Age	Mgm Fe per Kgm	Radioactivity counts per min 10	RBC 10^6	Hb Gm	Retic Peak %	MCV	MCHbC	Remarks
———	EM	68	2	900	124	61	366	162	25	Had been on Feosol
	RF	39	1	133	255	92	203	128	30	
-	AR	71	2	530	174	65	596	126	30	Diabetes
	LM	73	2	1080	128	56	204	132	33	
———	MP	58	1	260	276	124	77	138	33	Fe given before and after liver

Fig 7 Absorption of Radioactive Iron by Patients with Pernicious Anemia

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To one of the patients with hypochromic anemia (J L table 2) 1345 mg of inert iron as colloidal ferric hydroxide were given intravenously This amount was

recognized the isotope method is still the best one available for studies of iron absorption Recovery of unabsorbed iron from feces as was done in these studies is too difficult and too time consuming to be practical An error of approximately 10 per cent furthermore is involved in the recovery If a study is to be made of the relative absorption from different iron salts or of the effect of various influences like achlorhydria food the calcium phosphorus ratio etc on iron absorption the ideal procedure would be to use the isotope technic but to select only afebrile iron-deficient patients as test subjects

Objection might possibly be made to the experiments reported in this paper on the ground that one cannot be sure that a portion of the iron recovered in feces had not been absorbed and promptly excreted into the colon There is abundant evidence however that the amounts of iron excreted into the gastrointestinal tract are minute [14, 15] Any error introduced in this manner would be much less than the error of recovery per se and would be insignificant

These results are of theoretic interest chiefly as they relate to the theory that the intestinal mucosa serves as a major regulator of iron metabolism [5, 16, 17] Granick has postulated the following explanation of absorption the intestinal mucosal cells contain a protein apoferritin which combines with iron to form ferritin The ferritin iron is thought to be in equilibrium with small amounts of ferrous ions in the cells and the ferrous ions in turn are postulated as being in equilibrium with the iron in plasma According to this concept iron is taken up by mucosal cells until all the apoferritin is converted into ferritin No more is absorbed until some of the ferritin has given up its iron to plasma This theory explains beautifully the fact that iron-deficient subjects absorb more iron than do normal persons It does not account adequately however for the equally clear demonstration that patients with pernicious anemia in relapse with refractory anemia or with hemolytic anemias may also absorb fairly large amounts of the metal even though their tissues are replete with iron If the intestinal mucosa is a major regulator of iron metabolism protecting the body from an uptake sufficiently great to cause toxic concentrations in the tissues it at least is not as complete a regulator as it was first thought to be

Many of the factors which control iron absorption are unquestionably still unknown In unpublished experiments the authors have confirmed Hahn s observations that anemia by itself does not influence iron absorption Uptake from the intestinal tract has been shown to be independent of the plasma iron concentration On the other hand if tissue iron reserves of subjects with hypochromic anemia are partially restored by the parenteral administration of large amounts of iron uptake of the metal from the alimentary tract becomes less complete The possibility has been explored that a factor may be present in the blood of iron-deficient subjects which stimulates absorption but in two unpublished experiments the infusion of large amounts of plasma from iron-deficient into normal dogs has failed to affect the quantity absorbed The mucosal block theory of Hahn and Granick remains the best explanation for all the known facts about iron absorption but the block should be thought of in relative terms

In other experiments done on dogs made chronically anemic by regular phlebotomy the authors have demonstrated that the level of iron in the serum has no apparent effect on iron absorption If the serum iron level was raised above 500 mg per cent by intravenous administration of a soluble iron salt immediately before radioiron was given orally absorption wa not decreased (figure 9)

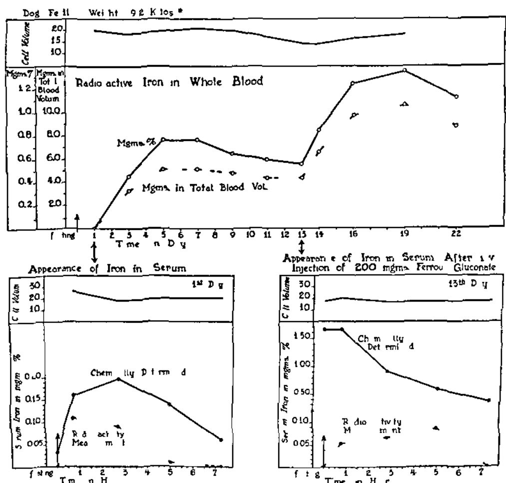


Fig 9 Effect of Iron in Blood Serum on Iron Absorption Radioactive Isotope Used

Discussion

The results of these experiments indicate clearly that patients with impaired hemoglobin formation are capable of absorbing more iron than is used for hemoglobin synthesis The data also suggest but do not prove that normal subjects may occasionally absorb more iron than is built immediately into hemoglobin Caution must be exercised therefore in interpreting estimates of iron absorption obtained solely by measuring the per cent of a given test dose of radioiron which appears in the circulating blood as hemoglobin Even when these limitations are

recognized the isotope method is still the best one available for studies of iron absorption Recovery of unabsorbed iron from feces, as was done in these studies is too difficult and too time consuming to be practical An error of approximately 10 per cent furthermore is involved in the recovery If a study is to be made of the relative absorption from different iron salts or of the effect of various influences like achlorhydria food the calcium phosphorus ratio etc on iron absorption the ideal procedure would be to use the isotope technic but to select only afebrile iron-deficient patients as test subjects

Objection might possibly be made to the experiments reported in this paper on the ground that one cannot be sure that a portion of the iron recovered in feces had not been absorbed and promptly excreted into the colon There is abundant evidence however that the amounts of iron excreted into the gastrointestinal tract are minute [11, 18] Any error introduced in this manner would be much less than the erro of recovery per se and would be insignificant

These results are of theoretic interest chiefly as they relate to the theory that the intestinal mucosa serves as a major regulator of iron metabolism [8, 16, 17] Granick has postulated the following explanation of absorption the intestinal mucosal cells contain a protein apoferritin which combines with iron to form ferritin The ferritin iron is thought to be in equilibrium with small amounts of ferrous ions in the cells and the ferrous ions in turn are postulated as being in equilibrium with the iron in plasma According to this concept iron is taken up by mucosal cells until all the apoferritin is converted into ferritin No more is absorbed until some of the ferritin has given up its iron to plasma This theory explains beautifully the fact that iron-deficient subjects absorb more iron than do normal persons It does not account adequately however for the equally clear demonstration that patients with pernicious anemia in relapse with refractory anemia or with hemolytic anemias may also absorb fairly large amounts of the metal even though their tissues are replete with iron If the intestinal mucosa is a major regulator of iron metabolism protecting the body from an uptake sufficiently great to cause toxic concentrations in the tissues it at least is not as complete a regulator as it was first thought to be

Many of the factors which control iron absorption are unquestionably still unknown In unpublished experiments the authors have confirmed Hahn s observations that anemia by itself does not influence iron absorption Uptake from the intestinal tract has been shown to be independent of the plasma iron concentration On the other hand if tissue iron reserves of subjects with hypochromic anemia are partially restored by the parenteral administration of large amounts of iron uptake of the metal from the alimentary tract becomes less complete The possibility has been explored that a factor may be present in the blood of iron-deficient subjects which stimulates absorption but in two unpublished experiments the infusion of large amounts of plasma from iron-deficient into normal dogs has failed to affect the quantity absorbed The mucosal block theory of Hahn and Granick remains the best explanation for all the known facts about iron absorption but the block should be thought of in relative terms

Summary and Conclusions

1 The isotope technic for studying iron absorption has been extended to measure the unabsorbed isotope in feces as well as the amount synthesized into hemoglobin The recovery of radioiron from feces was shown to be accurate within 10 per cent

2 There was suggestive evidence to indicate that with the 1 mg per kilogram dose employed normal subjects may sometimes absorb more iron than is converted within a two week period into hemoglobin

3 Patients with fever untreated pernicious anemia, and refractory anemia were shown to absorb more iron than they use for hemoglobin

4 Patients with hemolytic anemia may absorb more iron than can be recovered in the peripheral blood at any one time because isotopic hemoglobin is removed from the circulation at a rapid rate

5 Except in afebrile patients with hypochromic anemia acceptance of the per cent of a given dose of radioiron which appears in circulating hemoglobin as a measure of iron absorption must be made with caution

6 The theory that mucosal cells accept iron for absorption or block its assimilation provides the best known explanation for iron absorption but patients with adequate iron stores may assimilate considerable quantities of the metal and the block must be regarded as relative

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STUDIES ON FREE ERYTHROCYTE PROTOPORPHYRIN PLASMA IRON AND PLASMA COPPER IN NORMAL AND ANEMIC SUBJECTS*

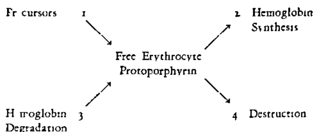
By G E Cartwright M D C M Huguley Jr M D Helen Ashenbrucker B A Jane Fay, M D and M M Wintrobe M D Ph D

THE VARIOUS anemias have been studied and classified clinically morphologically therapeutically and to a less extent etiologically but comparatively few chemical studies have been made Investigation of the chemical changes accompanying the various types of anemia offers a new approach to their study and gives rise to the hope that as the specific defects are elucidated their correction will become simplified The purpose of this paper is to present data on free erythrocyte protoporphyrin plasma iron and plasma copper in normal subjects and in subjects with various types of anemia

Review of Literature

The presence of protoporphyrin free in erythrocytes in addition to that in the hemoglobin molecule was reported by van den Bergh and co workers[1] in 1928 and since then has been confirmed repeatedly [2-7] Grotepass[3] and subsequently Watson Grinstein and Hawkinson[6] demonstrated that this protoporphyrin is identical with the protoporphyrin of hemoglobin namely protoporphyrin 9 type III A logical assumption is that this protoporphyrin is an intermediate compound in the synthesis of hemoglobin and that the free material is found because it has not been utilized for hemoglobin synthesis

The amount of free protoporphyrin in the erythrocyte may be postulated as depending upon the relative rates of (1) synthesis of protoporphyrin from precursors (2) utilization of the formed protoporphyrin for hemoglobin synthesis (3) formation of protoporphyrin from hemoglobin in the intact erythrocyte if such occurs and (4) destruction of protoporphyrin if this takes place This may be represented diagramatically



Data have been presented elsewhere[10] which indicate that reticulocytes contain more free erythrocyte protoporphyrin than do mature red corpuscles It has been reported that the protoporphyrin content of the bone marrow is increased when the percentage of normoblasts is increased In experimental hemolytic anemias the erythrocyte protoporphyrin did not increase when blood destruction was maximal but rose instead during the regenerative phase when reticulocytosis was marked [10] It has been suggested that increased EP usually signifies uncompleted hemoglobin synthesis which may be the consequence

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Aided by a grant from the United States Public Health Service and by grants from the American Cancer Society on recommendation of the Committee on Growth National Research Council from the Upjohn Company and from Parke Davis and Company

of the liberation of immature cells or is due to iron deficiency or to factors interfering with the utilization of iron in the synthesis of hemoglobin as for example lead poisoning [8, 10]

The manner in which protoporphyrin is broken down is unknown. It has not been demonstrated that destruction can take place in the intact red cell without the porphyrin being first formed into hemoglobin. The intravenous injection of protoporphyrin is not followed by a significant increase in the excretion of either bilirubin or coproporphyrin in the dog [11] There is evidence however to indicate that under certain circumstances increased free erythrocyte protoporphyrin may result from degradation of hemoglobin in intact erythrocytes. It has been demonstrated that following sterile incubation of red cells for twenty four to forty-eight hours there is an increase in the erythrocyte protoporphyrin [8] Furthermore red cells taken from the splenic vein following splenic stasis were found to have a greater protoporphyrin content than the cells in the splenic artery [19] Although the demonstration that protoporphyrin is formed from hemoglobin in intact erythrocytes in vitro does not necessarily indicate that such a mechanism exists in vivo there is considerable evidence that hemoglobin degradation can take place within the red cell in vivo. It would seem however that this takes place through a bile pigment iron-native globin complex similar to or identical with the pseudohemoglobin of Barkan or the verdohemoglobin of Lemberg rather than by liberating protoporphyrin

The literature dealing with plasma iron and with iron metabolism in general was reviewed recently by one of us (GEC) [21] Plasma iron has been the subject of many investigations. Early work was unsatisfactory because of lack of reliable methods. Several satisfactory methods are now available [12, 3] It seems clear that plasma iron functions as transport iron. The amount of iron in the plasma is affected by the rate of absorption of iron the balance between that going to and from the tissues and the equilibrium between the amount used for hemoglobin formation and that coming from hemoglobin catabolism. Recent studies have shown that the iron in plasma is bound by a specific β_1 globulin (Fraction IV of Cohn) [2]

The copper content of plasma in anemic state has been studied very little. Until recently progress in the field of investigation was retarded by the lack of a simple reliable method. The normal serum copper content has been reported by several investigators to be approximately 70 to 160 µg per cent [1, 4] Elevated serum copper values have been reported in pregnancy[19, 4] and in infections accompanied by anemia [2, 22] Plasma copper values in other clinical states accompanied by anemia have not been reported except by Locke Main and Rosbach[23] and it has been shown that the method which they used is unreliable [4]

Keilin and Mann[24] isolated a copper protein compound of unknown function from serum as well as red cells to which they gave the name of haemocuprein. The significance of this compound is unknown

Methods

The hematologic methods used in this study have been described elsewhere [5] Hemoglobin was determined by the photoelectric oxyhemoglobin method using an Evelyn photoelectric colorimeter standardized by the Van Slyke procedure. Erythrocyte protoporphyrin determinations were made by the method of Grinstein and Watson [6] The plasma iron was measured according to the procedure of Kitzes Elvehjem and Schuette[13] or by the method of Barkan and Walker [11] Both methods gave excellent recoveries (95 to 103 per cent of added iron) The method of Cartwright Jones and Wintrobe[14] was followed for the determination of plasma copper. This method has been shown to be accurate within ±10 per cent

Values for erythrocyte protoporphyrin (EP) are expressed throughout the paper in µg per 100 ml of red blood cells. Plasma iron (PI) and copper (PCu) are expressed in µg per 100 ml of plasma. Ht refers to volume of packed red cells in ml/100 ml. MCV refers to mean corpuscular volume in cµ. MCH refers to mean corpuscular hemoglobin in µµg. MCHC refers to mean corpuscular hemoglobin concentration in per cent

STUDIES ON FREE ERYTHROCYTE PROTOPORPHYRIN, PLASMA IRON AND PLASMA COPPER IN NORMAL AND ANEMIC SUBJECTS*

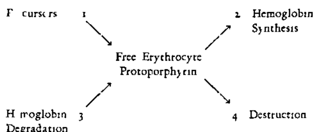
By G. E. CARTWRIGHT, M.D., C. M. HUGULEY, JR., M.D., HELEN ASHENBRUCKER, B.A., JANE FAY, M.D., AND M. M. WINTROBE, M.D., Ph.D.

THE VARIOUS anemias have been studied and classified clinically, morphologically, therapeutically and to a less extent etiologically, but comparatively few chemical studies have been made. Investigation of the chemical changes accompanying the various types of anemia offers a new approach to their study and gives rise to the hope that as the specific defects are elucidated their correction will become simplified. The purpose of this paper is to present data on free erythrocyte protoporphyrin, plasma iron, and plasma copper in normal subjects and in subjects with various types of anemia.

REVIEW OF LITERATURE

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The amount of free protoporphyrin in the erythrocyte may be postulated as depending upon the relative rates of (1) synthesis of protoporphyrin from precursors, (2) utilization of the formed protoporphyrin for hemoglobin synthesis, (3) formation of protoporphyrin from hemoglobin in the intact erythrocyte if such occurs, and (4) destruction of protoporphyrin if this takes place. This may be represented diagramatically:



Data have been presented elsewhere which indicate that reticulocytes contain more free erythrocyte protoporphyrin than do mature red corpuscles. It has been reported that the protoporphyrin content of the bone marrow is increased when the percentage of normoblasts is increased.[11] In experimental hemolytic anemias the erythrocyte protoporphyrin did not increase when blood destruction was maximal but rose instead during the regenerative phase when reticulocytosis was marked.[9] It has been suggested that increased EP usually signifies uncompleted hemoglobin synthesis which may be the consequence

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but only three values were above 57 μg In females the range was 16 to 140 μg but only one value was below 20 μg and only two were above 68 μg The difference between the geometric means for the two sexes is suggestive but not highly significant since the difference is only 1 7 times the standard error of the difference A slight skew is still present after attempted normalization by use of logarithms and it is our conclusion that the standard deviation given in table 1 is too large From inspection of the frequency distribution curve we have arbitrarily selected 13 to 55 μg per 100 ml of RBC as the normal range in males and 16 to 70 μg as the normal range in females No attempt has been made to determine in detail the variations

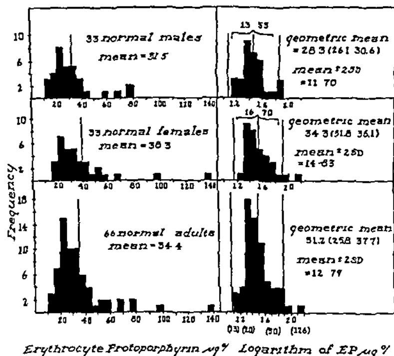


FIG 1 Distribution of normal values for erythrocyte protoporphyrin The graphs at the right are plotted in logarithmic values The vertical lines represent the mean +2 S D and −2 S D

in EP occurring over a period of time in individuals The variation was usually not marked *The variation in females was not related to the menstrual cycle* Simultaneous reticulocyte counts were not done

A total of ninety four determinations of plasma iron on 49 normal males and one hundred and two determinations on 43 normal females has been made The frequency distribution approximates a normal curve in each sex The means for the two sexes are almost identical and the standard deviations are of the same order As can be seen from table 2 the variation in normal values is great This seems to be a reflection of the variation in each individual In each of 3 males and 6 females from six to twenty one determinations were made over a period of six months to two years The means for these 9 individuals ranged from 68 to 140 μg per cent

Each of the three chemical determinations EP PI and PCu were done several or sometimes many times on each patient The values expressed in the tables are either representative or are means for the given case

Results

A *Normal values* The values for free erythrocyte protoporphyrin (EP) plasma iron (PI) and plasma copper (PCu) determined in normal adults are presented in tables 1 and 2 The subjects studied were not usually examined completely but they were apparently healthy Although the ages of the 68 men and 66 women varied from 20 to 63 years, there was a disproportionate number of young individuals

TABLE 1 —*Normal Subjects Erythrocyte Proptoporphyrin (μg/100 ml RBC)*

	Males	Females	Total
Observations	33	33	66
Range	13–79	16–140	13–140
Mean	32	39	35
Geometric Mean	28	34	31
Geometric Mean ± 2 S D	11–70	14 83	12 79

TABLE 2 —*Normal Subjects Plasma I on and Plasma Copper (μg per cent)*

	Pl sma Iron			Plasma Copp		
	M les	Femal	Total	M les	Fem les	Total
Observations	49	43	92	52	53	105
Range	43–210	28–202	28–210	86–161	87–161	86–161
Mean	105 1 ±4 3	104 3 ±5 5	104 [illegible] ±3 4	114 4 ±2 2	122 7 ±1 5	118 6 ±1 [illegible]
Standard Deviation	±30 3	±36 4	±32 8	±15 5	±10 6	±12 5
Mean ± 2 S D	45–166	32–177	39–170	83–145	10[illegible]–144	94–144

approximately two-thirds of each group being between 20 and 30 years of age However the data do not suggest that there is any variation related to age In many of the individuals examined several determinations were made of one or more of the three chemical constituents studied The figures analyzed in tables 1 and 2 represent a single value for each person usually the first obtained in the year 1947

A total of fifty six EP determinations in 33 males and fifty two determinations in 33 females has been made A frequency distribution curve of the determined values of erythrocyte protoporphyrin reveals a marked skew to the right (fig 1) By using the logarithm of each value a curve more nearly approaching the normal was obtained The analysis in table 1 was therefore carried out by this method In males the observed values ranged from 13 to 70 μg per 100 ml of red blood cells

but only three values were above 57 µg In females the range was 16 to 140 µg but only one value was below 20 µg and only two were above 68 µg The difference between the geometric means for the two sexes is suggestive but not highly significant since the difference is only 1 7 times the standard error of the difference A slight skew is still present after attempted normalization by use of logarithms and it is our conclusion that the standard deviation given in table 1 is too large From inspection of the frequency distribution curve we have arbitrarily selected 13 to 55 µg per 100 ml of RBC as the normal range in males and 16 to 70 µg as the normal range in females No attempt has been made to determine in detail the variations

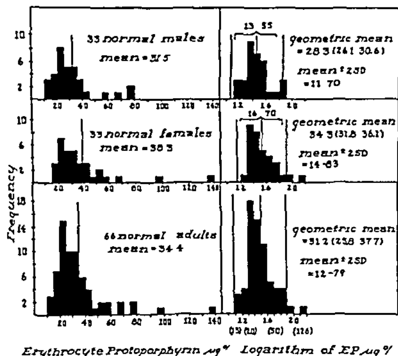


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However the variation in each individual was of the same order as that between the individuals Thus the average coefficient of variation in these 9 individuals was 40 per cent while that of the entire group was 31 per cent Because of the close approximation of the distribution of plasma iron values to the normal curve the mean value ±2 standard deviations has been used to indicate the normal No relationship was noted between the plasma iron levels in females and the menstrual cycle In one individual a series of determinations was made during the course of the day In this instance there was no definite trend the values from one morning to the next varying more than throughout the day No postprandial change was noted in this individual

TABLE 3 —*Pernicious Anemia*

Name	Age	Sex	RBC	Hgb	Ht	MCV	MCH	MCHC	Ret cs	E P	P I	P Cu
			Mill/c mm	Gm %	cc %	c μ	γγ	%	%	μg %	μg %	μg %
M B	74	F	1 75	6 4	18 5	106	37	35	0 4	30	222	100
A B	44	F	4 42	12 2	40 5	92	28	30	0 2	34	175	
R T	79	F	2 68	10 3	31 5	112	38	33	1 6	36	73	109
R Y	78	F	2 95	9 7	35 0	119	33	28	0 4	50	89	
S H	73	F	2 84	11 3	33 0	116	40	34	0 2	28	41	149
D M	46	F	2 35	9 5	26 5	113	40	36	1 9	41	149	115
H M	72	F	1 28	4 6	16 0	125	36	29	2 0	17	155	
L P	77	F	3 15	11 5	34 5	110	36	33	0 4	33	48	137
A S	82	F	2 70	9 7	28 5	105	36	34	0 2	26	113	128
M S	80	F	1 45	5 9	18 0	1–4	41	33	3 2	17	167	
T T	64	M	1 43	6 5	18 5	130	45	35	0 1	34	111	86
S S	69	M	1 50	5 9	17 8	119	39	33	3 2	9	282	
A G	74	M	1 34	4 9	16 0	120	36	30	1 8	44	250	183
C S	61	M	0 92	4 1	12 0	130	45	34	0 8	37	114	185
J W	60	M	1 31	4 6	15 0	116	35	31	1 2	—	222	—
N F	79	F	1 54	5 4	18 0	117	35	30	3 0	—	252	—
L R	26	F	1 54	4 5	17 5	113	29	26	1 5	—	28	—
J S	54	M	2 11	9 2	26 8	127	43	36	0 2	34	300	135
A B	69	M	1 38	5 1	14 4	104	37	35	2 0	29	201	108
N B	67	M	1 99	8 0	23 4	118	40	34	1 0	36	129	140

A total of seventy five determinations of plasma copper on 52 normal males and seventy five determinations on 53 females has been made The frequency distribution curve in males approximates a normal curve but in females though the range is the same as in males most of the values are grouped close to the mean The mean for the females is significantly higher than that for males The variation was not great the coefficient of variation being 13 6 per cent for males 8 6 per cent for females and 9 5 per cent for the group as a whole The coefficient of variation in 4 males and 5 females in each of whom five determinations were made was 5 4 to 16 1 per cent with an average of 10 9 per cent The mean value ±2 standard deviations has been used to indicate the limits of normal

B *Pernicious Anemia* The results obtained in 20 patients with pernicious anemia in relapse are shown in table 3 Erythrocyte protoporphyrin determinations were

made on 17 patients In 16 of these the values were within the normal range and in one patient (S S) the values were low repeatedly The mean for the entire group was 31 µg per 100 ml of red blood cells as compared with the normal of 35 µg (table 1) One patient (R T) is of particular interest This patient was admitted to the hospital on November 15 1945 in relapse at which time the EP was 36 µg She responded well to folic acid therapy but failed to return and received no further therapy She was readmitted in relapse one year later with fever and sub

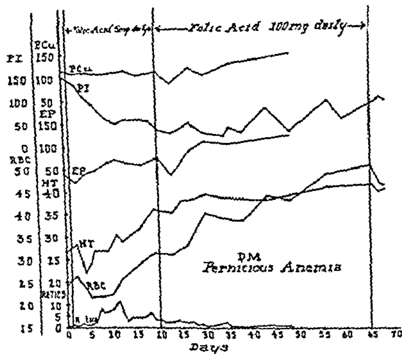


FIG 2 Pernicious anemia in relapse (case D M) treated with *folic acid* orally During therapy hypoferremia developed and there was a gradual rise in EP to above normal There was no significant change in plasma copper

PCu refers to plasma copper expressed in µg per cent PI to plasma iron expressed in µg per cent EP free erythrocyte protoporphyrin expressed in µg per 100 ml of red cells Ht volume of packed red cells in ml per 100 ml RBC red cell count in millions per c mm The reticulocytes are expressed as per cent of red cells

acute bacterial endocarditis On the second admission the volume of packed red cells was 19 ml per 100 ml the mean corpuscular volume 110 cµ and the EP 290 µg although the plasma iron was 268 µg per cent Thus it seems patients with pernicious anemia in relapse are at least in the presence of infection capable of synthesizing excess protoporphyrin In those patients who showed a satisfactory response to liver extract or folic acid therapy there was a marked rise in EP above normal during the reticulocytosis As the reticulocytosis subsided there was a fall in EP This is not demonstrated in figures 2 3 and 4 since the patients were given suboptimal doses of folic acid and a marked reticulocytosis did not occur A sharp

rise in EP associated with reticulocytosis has been observed in other patients treated with optimal doses of liver or folic acid [10] As demonstrated in figures 2 3 and 4 as the anemia was relieved there was a gradual rise in EP to the upper limits of normal In table 4 values for EP are presented for patients in relapse and remission In remission the EP tended to be in the upper limits of normal or even above normal It is of interest that EP in remission was always higher than during relapse

Plasma iron determinations have been made on 28 patients with pernicious anemia in relapse The data on 20 of these patients are presented in table 3 Of the

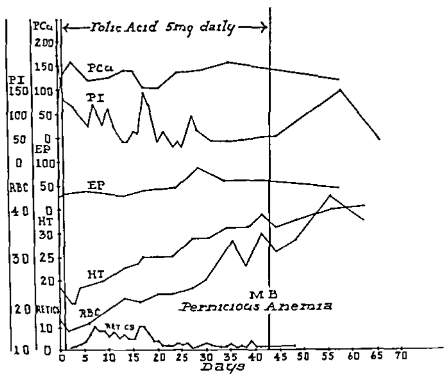


Fig 3 Pernicious anemia in relapse (case M B) treated with folic acid orally 5 mg per day During therapy slight hypoferremia developed and there was a rise in erythrocyte protoporphyrin There was no significant change in the plasma copper For symbols see figure 2

28 patients in 16 (57 per cent) the values were within the normal range in 11 (39 per cent) there was an elevation of the plasma iron and in one patient the value was below the normal Whatever the initial plasma iron may have been hypoferremia developed during the stage of rapid blood regeneration following therapy This is illustrated in figures 2 and 3 In many the hypoferremia persisted into the remission (table 4)

Plasma copper determinations have been made in 12 patients with pernicious anemia in relapse (table 3) The mean for the entire group was 137 µg per cent In 9 patients the values were within the normal limits and in 3 hypercupremia was noted During therapy and in remission (table 3) there was no consistent trend in

either direction although in the one patient with an initial low normal value there was a significant rise from 86 µg to 125 µg

C Iron Deficiency The results obtained in 13 patients with varying degrees of anemia due to iron deficiency are presented in table 5 Erythrocyte protoporphyrin determinations were made on all 13 and in each case the value was found to be above the normal The mean for the group was 207 µg per 100 ml of red blood cells No correlation between the degree of microcytosis or the degree of hypochromia and the magnitude of EP increase was noted There was a rough correlation between the duration of the anemia and the level of EP During the first

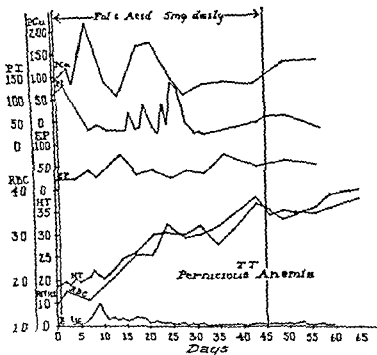


Fig 4 Pernicious anemia in relapse (case T T) treated with folic acid orally 5 mg per day During therapy a slight hypoferremia developed and there was a gradual increase in protoporphyrin There were rather large fluctuations in plasma copper For symbols see figure 2

10 to 20 days following the onset of iron therapy that is during the period of reticulocytosis there was a slight increase or no change at all in the EP Then as the volume of packed red cells mean corpuscular volume and mean corpuscular hemoglobin concentration returned to normal the EP diminished This reached normal only some time after the blood had returned to normal (figs 5 6 7)

Plasma iron determinations were made in 13 patients The results are presented in table 5 and are uniformly low The mean for the entire group is 23 µg per cent as compared with the normal of 105 µg During therapy (figs 5 6 7) there was a slow rise in plasma iron but not until some time after the blood had returned to normal did the plasma iron reach normal

TABLE 4 —*Pernicious Anemia before and after Therapy*

Patient	P iod	Ht	E P	P I	P C
		cc %	µg %	µg %	µg %
M B	Relapse	19	30	222	100
	Remission	34	60	108	131
S H	Relapse	33	28	41	149
	Remission	40	73	37	148
D M	Relapse	26	41	41	115
	Remission	42	116	45	140
L P	Relapse	35	33	48	137
	Remission	38	44	31	—
A S	Relapse	28	26	113	128
	Remission	39	36	60	143
M S	Relapse	18	17	167	—
	Remission	44	52	62	—
T T	Relapse	18	34	111	86
	Remission	40	78	23	125
S S	Relapse	18	9	282	—
	Remission	41	5/	111	—
A G	Relapse	16	44	250	183
	Remission	43	88	36	—

TABLE 5 —*I on D ficiency*

Name	Ag	Se	RBC	Hgb	Ht	MCV	MCH	MCHC	R t c	E P	P I	P C
			M ll mm	Gm %	%	µ	γγ	%	%	µg %	µg %	µg %
E Mc	66	F	3 47	5 4	21 0	60	16	26	1 0	475	16	
H R	23	M	3 95	5 8	23 2	59	15	25	—	389	15	
G A	60	M	3 13	4 8	21 0	67	15	23	4 0	183	25	17
V C	50	F	5 30	8 4	34 0	62	15	25	0 2	99	30	128
J H	33	F	4 /0	9 /	35 0	74	21	28	0 5	100	31	
J H	71	F	5 01	7 8	29 5	59	16	26	1 7	216	20	210
G J	73	M	3 32	7 2	32 5	92	20	22	1 4	166	21	
H W	65	F	4 84	7 /	29 0	59	16	26	4 0	319	15	130
J J	49	F	4 88	9 0	35 0	2	18	26	3 0	220	19	145
D L	58	M	3 05	5 9	22 0	2	19	27	1 8	145	11	205
G L	45	F	4 38	7 2	31 0	0	6	23	1 0	100	32	
D H	49	M	4 47	8 7	32 6	73	20	27	1 6	200	27	155
J H	20	F	4 24	9 3	32 6	77	22	28	0 6	184	31	183

Plasma copper determinations were made in 8 patients (table 5) In 6 the values were found to be high and in 2 the values were within normal limits No cor

relation could be found between the degree of anemia and the degree of hyper cupremia Plasma copper was followed during therapy in one patient (fig 7) In this patient there was a fall in the copper which began about twenty days after the onset of therapy and reached normal at about the time the blood returned to normal and before the plasma iron or EP had reached normal

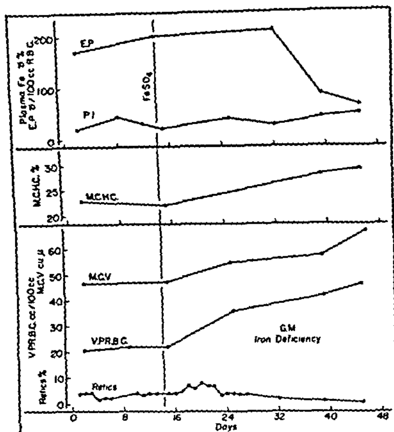


Fig 5 Iron deficiency anemia (case G M) with hypoferremia and an increase in EP Treatment with ferrous sulfate was followed by a significant though delayed fall in EP and a gradual increase in plasma iron as the anemia was relieved V P R B C refers to volume of packed red cells in ml per 100 ml (Ht) MCV to mean corpuscular volume in c μ MCHC mean corpuscular hemoglobin concentration in per cent For other symbols see figure 2

D *Anemia of Infection* As previously reported the anemia associated with chronic infection is accompanied by an elevated erythrocyte protoporphyrin low plasma iron and high plasma copper The results in 10 patients not previously recorded are presented in table 6 These data confirm our previous findings The erythrocyte protoporphyrin was elevated in 9 of the 10 patients hypoferremia and hypercupremia were present in all those so examined A number of additional patients with acute and subacute infections have been studied and data are now available concerning the rapidity and sequence of the changes in relation to the onset of the disease and during convalescence

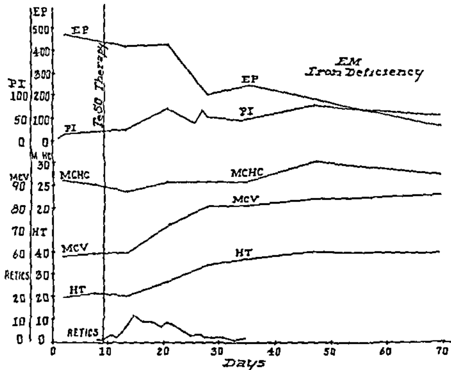


FIG 6 Iron deficiency anemia (case E M) with hypoferremia and an increase in EP During therapy with ferrous sulfate there was a significant decrease in EP and a gradual rise in plasma iron The volume of packed red cells and indices reached normal before the EP returned completely to normal For symbols see figures 2 and 5

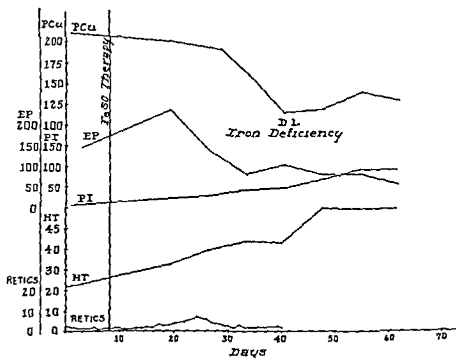


FIG 7 Iron deficiency anemia (case D L) with hypoferremia hypercupremia and increase in EP During therapy with ferrous sulfate a marked drop in plasma copper took place There was an initial increase in EP followed by a drop to normal The plasma iron rose gradually For symbols see figure 2

In acute infections with fever such as uncomplicated lobar pneumonia bacterial meningitis pharyngitis scarlet fever and otitis media hypoferremia was found to develop within a few days after the onset of fever This is illustrated in 2 patients with meningococcal meningitis (fig 8) When the disease was of short duration the plasma iron rose when the fever subsided and no anemia developed Hypoferremia appears to be the first change to take place and occurs in a variety of conditions associated with fever In figure 9 the effect of induced fever (typhoid vaccine) on the plasma iron is demonstrated With each paroxysm the iron dropped markedly and returned rapidly to normal shortly after the cessation of fever No anemia developed and there was no significant change in plasma copper or rise in erythrocyte protoporphyrin

TABLE 6 —Chronic Infections

Name	Age	Sex	Disease	Duration	RBC	Hgb	Ht	MCV	MCH	MCHC	E P	P I	P Cu
J W	28	M	Subacute Bacterial Endocarditis	9	3 82	8 9	28 5	75	23	31	266	25	175
D S	35	F	Subacute Bacterial Endocarditis	9	3 35	7 7	29 8	89	23	26	137	—	258
T M	,8	M	Disseminated Tuberculosis	12	—	10 0	31 5			31	128	36	159
R M	53	M	Lung Abscess	8	3 25	7 0	2 0	83	22	26	564	30	267
J L	63	M	Osteomyelitis	24	3 82	9 0	30 2	79	24	30	221	17	—
A B	54	M	Pneumonia	1	4 26	10 5	33 0	78	25	32	91	15	169
T E	45	M	Actinomycosis		4 34	11 8	39 0	90	25	28	63	14	179
A R	45	M	Lung Abscess	1	3 36	10 5	32 2	96	34	36	345	27	199
J K	69	F	Empyema	3	3 76	11 4	35 2	93	30	32	100	20	189
I C	36	F	Empyema	1	4 28	11 7	35 0	82	27	33	55	30	214

The effect on the plasma iron of a more prolonged illness is demonstrated in figures 10 and 11 In patient W V (fig 10) there was an initial hemolytic phase with slight jaundice accompanying lobar pneumonia The total serum bilirubin was 1 5 mg per cent With this there was an initial hyperferremia which was followed by the hypoferremia usually associated with infection This persisted for about twenty five days without anemia developing Patient I C (fig 11) a woman 36 years of age who had pneumonia and an acute lung abscess was seen on about the twenty first day of illness The plasma iron at this time was 30 μg per cent and the volume of packed red cells 35 ml The disease responded rapidly to parenteral penicillin therapy and the plasma iron rose promptly Two patients (F L pyonephrosis and D S empyema) with more chronic illnesses are described in figures 12 and 13 Significant anemia was present in both The plasma iron did not reach normal in either patient until after the anemia disappeared

In acute infections with hypoferremia no change in EP has been noted This is illustrated in figures 8 9 and 11 Only when the infection persisted for a month or more or when significant anemia developed did a rise in EP occur This rise sometimes reached its maximum after the maximal development of anemia This is illustrated in figure 14 Return of EP to normal took place slowly (figs 10 12 13 and 14) and did not reach normal until long after the anemia had disappeared

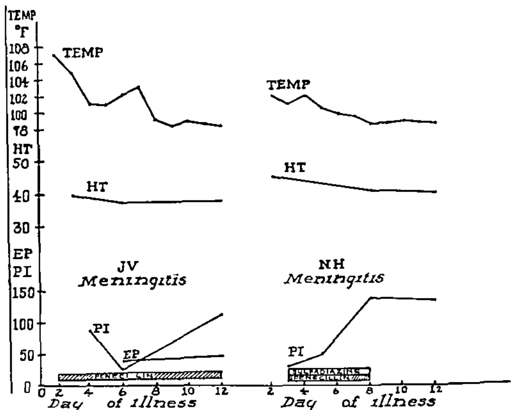


FIG 8 The effect of acute infections on the plasma iron as exemplified in two patients with meningococcal meningitis (J V and N H) Hypoferremia developed early in both patients The iron returned rapidly to normal as the f ver subsided No anemia developed and there was no increase in EP For symbols see figure 2

The rise in EP seemed to be correlated with the duration of the infection and to a less extent with the severity of the anemia

The rise in plasma copper took place some time after the development of hypoferremia but was observed before or in the absence of a rise in EP (fig 11) As can be seen in figure 9 no significant change took place in the plasma copper during the paroxysms of fever In chronic infections with anemia (table 6) hypercupremia was almost invariably present The copper returned to normal mo e rapidly than did the EP or iron and became normal about the time of the disappearance of anemia as illustrated in figure 10

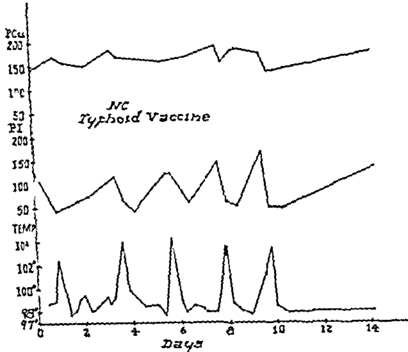


Fig 9 The effect of induced fever (typhoid vaccine) on the plasma iron With each paroxysm the plasma iron dropped markedly and returned to normal shortly after the cessation of fever There was no significant change in the plasma copper For symbols see figure 2

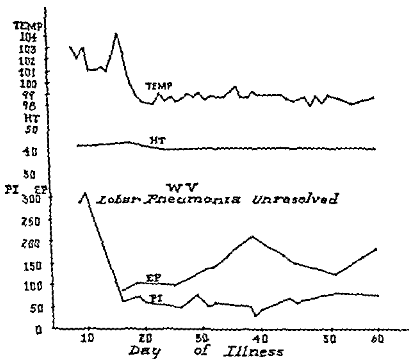


Fig 10 Unresolved lobar pneumonia (case W V) There was an initial hemolytic phase with jaundice bilirubinemia and hyperferremia Following this hypoferremia developed and a *rise* in EP occurred For symbols see figure 2

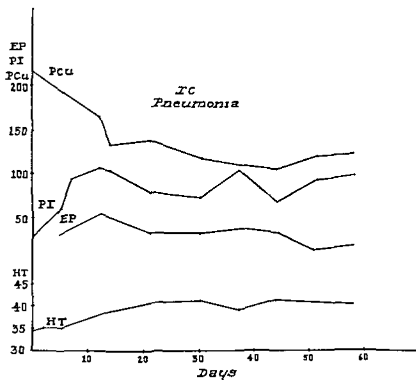


Fig 11 A patient with lobar pneumonia and early lung abscess (case I C) with anemia hypoferremia and hypercupremia which responded well to parenteral penicillin As the anemia disappeared the plasma iron rose and plasma copper returned to normal There was no significant change in EP For symbols see figure 2

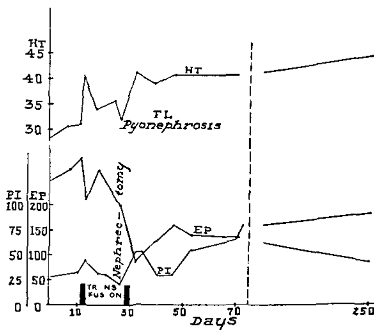


Fig 12 Chronic infection (pyonephrosis case F L) with anemia hypoferremia and an increase in EP Kidney function was not diminished Following nephrectomy the anemia disappeared the plasma iron rose and the EP decreased For symbols see figure 2

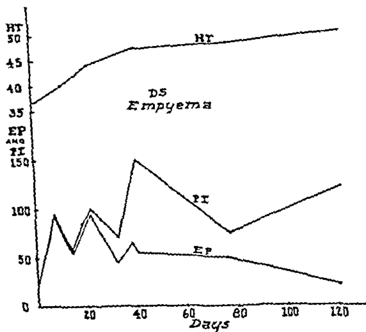


Fig 13 Chronic infection (empyema case D S) with anemia hypoferremia and an increase in EP As the infection subsided the EP diminished to normal and the plasma iron rose For symbols see figure 2

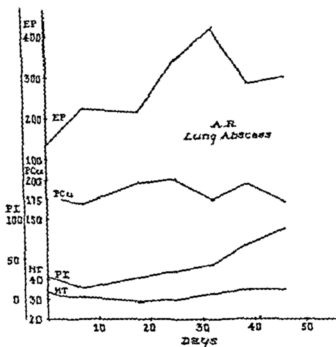


Fig 14 Chronic infection (lung abscess case A R) with anemia hypoferremia hypercupremia and an increase in EP The hypoferremia and hypercupremia developed early The maximal rise in EP occurred later at the time when the anemia was most severe *This patient was unusual in that the plasma iron rose in spite of persistent anemia* For symbols see figure 2

It is interesting that one patient with anemia and chronic osteomyelitis de veloped severe nephritis during the course of his illness The serum albumin fell to 1 73 grams per cent and the serum globulin to 1 79 grams per cent At the same time the plasma copper dropped from 246 μg per cent to 40 μg per cent and remained at this low level

E *Anemia of Nephritis* The results in 10 patients with anemia associated with various types of nephritis are presented in table 7 In 8 of the 10 patients the erythrocyte protoporphyrin was elevated and in 2 the values were normal In 4 of the patients the plasma iron was low and in 6 normal Plasma copp-r de terminations were made on 5 patients In 4 the values were high and in the remain ing patient the value was normal Sufficient study has not b-en made to correlate

TABLE 7 —Neph i s

Name	Age	Sex	Typ	RBC	Hgb	Ht	MCV	MCH	MCHC	R tic	E P	PI	P Cu	BUN	T P	Alb	Gl b
				m l l mm	Gm p ce t	c c	c μ	γγ	c	c	μg	μg	μg c	mg c	Gm c	Gm c	Gm c
A B	73	F	Chronic Arte l t c	2 84	8 1	24 0	85	29	34	4 8	138	32	240	51	6 5	4 2	2 3
D H	18	M	Chronic Gl meru l ph itis	3 0	7 5	29 0	95	24	24	—	80	21	136	27	4 8	2 5	2 3
R C	69	M	Embol c S pp a t	2 2	7 2	26 5	75	26	35	2 8	9	21	—	35	5 6	2 9	2 7
G C	50	M	M l g nt Nephro s le s s	3 50	9 5	31 1	90	27	31	—	71	35	193	91	7 3	4 6	2 7
C C	34	M	Chro Gl m lon phrit	3 3	10 0	30 2	91	30	33	1 2	178	6	—	75	—	—	—
J A	60	M	Ch Gl me u l ph itis	2 33	7 2	20 5	88	31	35	—	101	111	165	154	5 8	4 0	1 8
A M	39	F	Ch Glome u l neph tis	2 25	7 0	0 5	91	31	34	—	59	117	183	66	7 6	4 2	3 4
I M	37	F	Ch Pyelo e ph t	3 44	9 5	3 0	93	28	30	1 8	18	81	—	21	5 1	2 7	2 4
C J	73	M	Neph ler s	2 15	5 5	18 0	84	26	31	1 4	198	1	—	51	4 7	2 4	2 3
A J	63	M	Neph l s	5 40	15 0	48 5	90	28	31	—	100	115	—	20	—	—	—

these chemical changes with the type of nephritis nor with the other metabolic changes which take place in this disease

F *Lymph node disorders and Leukemia* Observations in 19 patients with various types of lymph node disorders and leukemia are presented in table 8 All determinations were made prior to treatment with x ray nitrogen mustard or urethane Erythrocyte protoporphyrin determinations were made on 19 patients In 12 patients the values were elevated and in 7 normal Plasma iron determinations were made on 19 patients In 12 patients the values were normal in 6 low in 1 elevated Deter minations of plasma copper were made in 6 patients and values greater than normal were found in all The rise in EP was more marked in the lymphoma group than in the leukemia group Hypoferremia was not observed in the leukemia group

G *Thalassemia* Observations in 6 patients with thalassemia minor and 5 patients with thalassemia major are presented in table 9 Unfortunately EP determinations

could *not* be carried out except in one patient In all 6 of the patients with the minor variant of the disease the serum iron was normal In 4 of the 6 hyper

T BLE 8 —*Lymph Nod Disorders and Leukemia*

Name	Ag	S x	D se se	RBC	Hgb	Ht	MCV	MCH	MCHC	FP	PI	PCu	Comm t
				M c mm	Gm %	c	c μ	γγ	c	μg%	μg%	μg%	
C. B	25	M	Hodgkin s Disease	4 25	10 6	34 0	80	25	31	121	21	—	—
R F	5	M	Hodgkin s Disease	3 23	8 9	29 2	90	28	30	47	40	—	—
E G		F	Hodgkin s Disease	5 39	14 2	43 4	80	26	33	182	52	—	—
F H	40	M	Hodgkin s Disease	3 52	8 6	25 0	71	24	34	61	31	—	—
M P	27	F	Hodgkin s Disease	2 42	5 1	20 0	83	21	26	322	36	—	Retics 7 4%
W R	57	M	Hodgkin s Disease	3 12	6 8	25 0	80	22	2	200	56	—	—
A S	34	M	Hodgkin s Disease	2 89	8 4	30 5	105	29	30	79	2	199	—
N S	26	F	Hodgkin s Disease	3 10	5 3	20 0	65	17	26	115	70	—	—
H S	21	F	Hodgkin s Disease	4 50	11 5	38 5	85	28	30	58	29	212	—
M T	36	F	Hodgkin s Disease	3 69	11 3	32 2	8	31	35	94	34	—	—
C W	24	M	Hodgkin s Disease	4 25	9 8	32 5	,	23	30	98	16	222	—
P K	56	M	Reticulum Cell Sarcoma	4 25	9 7	34 0	80	23	29	236	23	165	—
M G	36	F	Reticulum Cell Sarcoma	5 24	11 6	37 8	71	22	31	51	15	—	—
F M	33	F	Chron c Myelocytic L	2 40	6 0	18 0	75	25	33	114	94	—	W BC 465 000
M M	58	F	Chronic Myelocytic L.	4 00	12 0	36 0	90	30	33	40	46	—	W BC 160 000
C C	79	F	Chronic Myelocytic L	4 67	12 7	40 5	87	27	31	48	45	173	W BC 24 750
G W	56	F	Chronic Myelocytic L	3 84	9 5	28 5	74	25	33	65	60	1,4	W BC 151 000
H R	56	M	Chronic Lymphocytic L	1 61	4	13 0	81	29	36	61	292	—	W BC 37 000
G C	25	M	Acute Lymphoblastic L	4 95	14 6	43 0	87	29	34	26	133	—	W BC 39 000

TABLE 9 —*Thalassemia*

Name	Ag	S	Type	RBC	Hgb	Ht	MCV	MCH	MCHC	Ret cs	PI	PCu
				Mill c mm	Gm %	cc %	μ	γγ	%	%	μg%	g%
J T	45	F	Minor	6 34	11 8	36 5	58	19	32	1 4	103	146
A A	28	F	Minor	5 52	10 5	34 0	61	19	31		144	111
K Z	46	F	Minor	5 25	11 7	35 5	68	22	33		76	138
M D	41	F	Minor	5 47	12 9	37 2	68	24	35		90	226
M C	65	F	Minor	5 92	12 6	37 0	63	21	34		176	218
J T	31	F	M nor	6 05	11 9	35 0	58	20	34		110	153
P F	9	M	Major	3 15	7 8	23 0	73	25	34	4 9	202	181
V P †	1	M	Major	3 35	5 8	26 0	77	17	22	19 0	80	330
S F	2	F	Major	2 15	4 2	14 0	65	20	30	5 0	211	162
A P	7	M	Major	2 09	4 3	14 2	68	21	30	2 2	310	—
N P	7	M	Major	1 97	4 0	13 2	67	20	30	3 2	288	—

EP 65 μg
† Infection (T 101)

cupremia was present In thalassemia major hyperferremia was present in 4 of the 5 patients In the fifth an infection with fever was present and the iron was within

It is interesting that one patient with anemia and chronic osteomyelitis de veloped severe nephritis during the course of his illness The serum albumin fell to 1 73 grams per cent and the serum globulin to 1 79 grams per cent At the same time the plasma copper dropped from 246 μg per cent to 40 μg per cent and remained at this low level

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TA LE 7—Neph i s

Name	Age	Sex	Type	RBC	Hgb	Ht	MCV	MCH	MCHC	Re t c	E P	P I	P Cu	BUN	T P	Alb	Gl b
				m l l mm	Gm p l	cc c	μ	γγ	c	c	μg c	μg	μg	mg c	Gm c	Gm c	Gm c
A B	73	F	Chronic Art io scle ot c	2 84	8 1	24 0	85	29	34	4 8	138	32	240	51	6 5	4 2	2 3
D H	18	M	Chronic Glom ru l eph it s	3 05	7 5	29 0	9	24	24	—	80	21	13(	27	4 8	2 5	2 3
R C	69	M	Fmbol c Suppura ti e	2 72	7 2	26 5	5	26	35	2 8	9	21	—	35	5 6	2 9	2 7
G C	50	M	M l g t Nephro sclerosis	3 50	9 5	31 1	90	27	31	—	71	35	193	91	7 3	4 6	2 7
C C	34	M	Chronic Clomeru lonephritis	3 3	10 0	30 2	91	30	33	1 2	1 8	6	—	75	—	—	—
J A	60	M	Ch n c Glom loneph t	2 33	7 2	20 5	88	31	35	—	101	111	165	154	5 8	4 0	1 8
A M	39	F	Ch o c Gl m l ph t	2 25	7 0	20 5	91	31	34	—	59	117	183	66	7 6	4 2	3 4
I M	37	F	Ch Py lon ph t s	3 44	9 5	3 0	93	28	30	1 8	18	81	—	21	5 1	2 7	2 4
C J	73	M	N ph oscle osis	2 15	5 5	18 0	84	26	31	1 4	198	15	—	51	4 7	2 4	2 3
A J	63	M	Neph o cl osis	5 40	15 0	49 5	90	28	31	—	100	115	—	20	—	—	—

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TABLE 8 —*Lymph Node D sord s and Leukemia*

Name	Age	Sex	Disease	RBC	Hgb	Ht	MCV	MCH	MCHC	FP	PI	PC	Comment
				M / mm	Gm %	%	cμ	γγ	%	μg%	μg%	μg%	
C B	25	M	Hodgkin s Disease	4 25	10 6	34 0	80	25	31	121	21	—	—
R F	57	M	Hodgkin s Di ease	3 23	8 9	29 2	90	28	30	47	40	—	—
E G		F	Hodgkin s Disease	5 39	14 2	43 4	80	26	33	182	52	—	—
F H	40	M	Hodgkin s Disease	3 52	8 6	25 0	71	24	34	61	31	—	—
M P	27	F	Hodgkin s Disease	2 42	5 1	20 0	83	21	26	322	36	—	Retics 7 4%
W R	57	M	Hodgkin s Disease	3 12	6 8	25 0	80	22	2	200	56	—	—
A S	34	M	Hodgkin s Disease	2 89	8 4	30 5	105	29	30	79	72	199	—
N S	26	F	Hodgkin s Disease	3 10	5 3	20 0	65	17	26	115	70	—	—
H S	21	F	Hodgkin s Disease	4 50	11 5	38 5	85	28	30	58	29	212	—
M T	36	F	Hodgkin s Disease	3 69	11 3	32 2	87	31	35	94	34	—	—
C W	24	M	Hodgkin s Disease	4 25	9 8	32 5	-	23	30	98	16	222	—
P K	56	M	Reticulum Cell Sarcoma	4 25	9 7	34 0	80	23	29	236	23	165	—
M G	36	F	Reticulum Cell Sarcoma	5 24	11 6	37 8	71	22	31	51	15	—	—
F M	33	F	Chronic Myelocytic L	2 40	6 0	18 0	75	25	33	114	94	—	WBC 465 000
M M	58	F	Chronic Myelocytic L	4 00	12 0	36 0	90	30	33	40	46	—	WBC 160 000
C C	79	F	Chronic Myelocytic L	4 67	12 7	40 5	87	27	31	48	45	1 3	WBC 24 750
G W	56	F	Chronic Myelocytic L	3 84	9 5	28 5	74	25	33	65	60	174	WBC 151 000
H R	56	M	Chronic Lymphocytic L	1 61	4 7	13 0	81	29	36	61	292	—	WBC 37 000
G C	25	M	Acute Lymphoblastic L	4 95	14 6	43 0	87	29	34	26	133	—	WBC 38 000

TABLE 9 —*Thalassemia*

Name	Ag	S	Type	RBC	Hgb	Ht	MCV	MCH	MCHC	Retics	PI	PCu
				Mill n c mm	Gm %	%	μ	γγ	%	%	μg%	μg%
J T	45	F	Minor	6 34	11 8	36 5	58	19	32	1 4	103	146
A A	28	F	Minor	5 52	10 5	34 0	61	19	31		144	111
K Z	46	F	Minor	5 25	11 7	35 5	68	22	33		76	138
M D	41	F	Minor	5 47	12 9	3 2	68	24	35		90	226
M C	65	F	Minor	5 92	12 6	37 0	63	21	34		1,6	218
J T	31	F	Minor	6 05	11 9	35 0	58	20	34		110	153
P F	9	M	Major	3 15	7 8	23 0	73	25	34	4 9	202	181
V P †	1	M	Major	3 35	5 8	26 0	77	17	22	19 0	80	330
S F	2	F	Major	2 15	4 2	14 0	65	20	30	5 0	211	162
A P	7	M	Major	2 09	4 3	14 2	68	21	30	2 2	310	—
N P	7	M	Major	1 97	4 0	13 2	67	20	30	3 2	288	—

EP 65 μg
† Infection (T 101)

cupremia was present In thalassemia major hyperferremia was present in 4 of the 5 patients In the fifth an infection with fever was present and the iron was within

the normal limits In the 3 patients with the major form of the disease in whom serum copper determinations were made hypercupremia was present in each

In figure 15 the negative effects of intravenously administered pyridoxine on the hyperferremia of two patients with thalassemia major are presented These same 2 patients had been previously reported as benefiting from combined prolan B and pyridoxine therapy [27]

H *Miscellaneous conditions* Observations in a variety of hematologic conditions other than those discussed above are presented in table 10 Several findings are

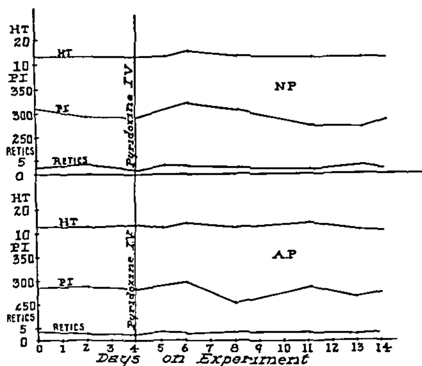


Fig 15 Two patients (N P and A P) with thalassemia major and hyperferremia The hyperferremia was not affected by intravenous administration of 50 mg pyridoxine hydrochloride daily For symbols see figure 2

worthy of comment In the 4 patients with aplastic anemia who were studied the plasma iron was markedly elevated in each Erythrocyte protoporphyrin determinations were made in 2 In one patient the value was slightly elevated and in the other normal Two patients with myelophthisic anemia were found to have high EP A patient with hemochromatosis had a normal plasma iron and a low plasma copper In 2 patients with plumbism there was a marked elevation of the EP *Sufficient data are not available on hemolytic anemia to draw conclusions since* only 2 patients were studied and the results differed

Discussion

Our values for the normal EP correspond with those which Watson Grinstein and Hawkinson[5] obtained in 12 normal individuals although the range in our

series is somewhat higher The data for normal plasma iron are similar to those recorded by others[16] except that whereas most observers have found that the mean value for females is significantly lower than for males in our series the means were

TABLE 10 —Miscellaneous Conditions

Name	Age	Sex	Condition	RBC	Hct	Hb	MCV	MCH	MCHC	Retic	EP	PI	PCu
				Mill/cmm	Gm%	%	µ³	γγ	%	%	µg%	µg%	µg%
D H	26	M	Aplastic Anemia[1]	2 50	9 8	28 0	112	39	35	0 2	46	318	169
P D	61	M	Aplastic Anemia[2]	1 96	5 4	16 5	84	28	33	0 1	70	260	120
L B	39	F	Aplastic Anemia[2]	2 54	7 5	22 0	87	30	37			226	
N G	35	F	Aplastic Anemia[3]	1 50	3 8	13 0	87	26	30	0 8		222	
E G	63	M	Myelophthisic Anemia[4]	1 69	3 4	13 5	80	28	35	3 4	143	1 5	
M G	67	F	Myelosclerosis[3]	2 7	9 5	31 8	111	34	30	9 6	138	143	
N A	15	M	Congenital Hemolytic Icterus[2]	3 29	11 5	34 6	105	35	33	4 8	45	35	
T A	27	F	Congenital Hemolytic Icterus[2]	3 02	11 0	29 0	96	36	38	11 2	149	88	115
V P	24	M	Plumbism	5 07	13 8	42 0	83	27	33	1 0	274	191	104
J D	25	M	Plumbism[3]	4 38	10 6	36 0	82	24	29	5 0	206	125	100
E P	25	M	Hemochromatosis	4 41	15 0	48 3	109	34	31		28	147	60
N P	68	M	Polycythemia	0	22 6	72 0	92	29	32		25	184	
J R	32	M	Polycythemia[5]	8 67	22 7	75 0	88	25	29		76	267	
J V	73	M	Polycythemia[6]	7 51	22 2	66 0	88	28	32	0 9	26	69	
F G	60	M	Polycythemia	7 25	17 7	60 0	83	25	30		22	125	
C D	70	F	Hypothyroidism[7]	4 10	12 0	33 0	80	27	36		52	77	111
A F	56	F	Hypothyroidism	4 33	11 3	36 0	83	26	31		80	75	
M S	55	F	Hypothyroidism	3 82	11 1	34 0	89	29	33		49	157	1 6
J O	61	M	Cirrhosis Liver	2 55	7 4	25 0	98	29	30	1 0	51	44	
T N	42	M	Cirrhosis Liver	3 48	10 4	32 0	92	32	33		80		
E P	19	F	Subacute Yellow Atrophy	4 27	12 5	39 0	91	29	32		40	108	122
L S	71	F	Banti s Syndrome[2]	3 68	12 5	32 0	87	34	39	1 4	35	99	
W G	57	M	Banti s Syndrome[2]	3 73	10 4	31 8	85	28	33	0 7	62	88	180
M P	72	F	Multiple Myeloma	3 68	11 1	35 0	95	30	32	0 1	46	46	
W W	46	M	Multiple Myeloma	3 81	10 8	32 5	85	28	33		53	69	135
C B	37	M	Constitutional Hyperbilirubinemia	5 84	16 4	45 6	83	30	36	0 2	26	101	129

[1] Hypoplastic marrow [2] Normoblastic marrow [3] Fibrotic bone marrow Carcinoma prostate
[5] Secondary Primary Diabetic

almost identical The normal plasma copper values also agree with those obtained by others using reliable methods [18–]

In table 11 the results of erythrocyte protoporphyrin plasma iron and plasma copper studies in a variety of clinical conditions associated with anemia are summarized In general it was found that in pernicious anemia in relapse the EP was

normal the plasma iron normal or high and the plasma copper usually normal Anemia due to iron deficiency and chronic infections was accompanied by an elevated EP, hypoferremia and hypercupremia In nephritis with anemia the EP was generally increased the plasma iron was low or normal and the plasma copper was increased Anemia associated with lymph node disorders or leukemia was accompanied by a normal or high EP a low or normal plasma iron and an increase in plasma copper In thalassemia minor the serum iron was normal hypercupremia was found in 4 out of 6 patients Thalassemia major was accompanied by both hyperferremia and hypercupremia Hyperferremia was present in all 4 patients with aplastic anemia who were studied In 2 patients with plumbism there was a marked increase in EP Hypocupremia was encountered only twice in one patient with severe nephritis and hypoalbuminemia and in one patient with hemochromatosis

TABLE 11 —*Summary of the Data*

Co d t on	Erythrocyte Pr t porphyrin				Plasm Iron				Pla m Copper			
	No Pts	Low	No mal	H gh	No Pts	Low	Nor mal	H gh	No Pts	Low	No m l	H gh
Pernicious Anemia	17	1	16	0	28	1	16	11	12	0	9	3
Iron Deficiency	13	0	0	13	13	13	0	0	8	0	2	6
Chronic Infections	10	0	1	9	9	9	0	0	9	0	0	9
Nephritis	10	0	2	8	10	4	6	0	5	0	1	4
Lymphoma or leukemia	19	0	7	12	19	7	11	1	6	0	0	6
Aplastic Anemia	2	0	1	1	4	0	0	4	2	0	1	1
Thalassemia Major					5	0	1	4	3	0	0	3
Thalassemia Minor	1	0	1	0	6	0	6	0	6	0	2	4
Hemochromatosis	1	0	1	0	1	0	1	0	1	1	0	0
Plumbism	2	0	0	2	2	0	1	1	2	0	2	0
Myelophthisic Anemia	2	0	0	2	2	0	1	1				
Hemolytic Anemia	2	0	1	1	2	1	1	0	1	0	1	0

In general it was found that in conditions characterized by hypoferremia the EP and plasma copper were elevated (anemia of infection iron deficiency nephritis lymph node disorders and leukemia) Analyzing the data in another way it would seem that there was an increase in EP in anemic stat s associated with a normoblastic bone marrow due to a disturbance in hemoglobin synthesis i e iron deficiency anemia of infection nephritis lead poisoning and some cases of lymphoma and leukemia In contrast in pernicious anemia which is characterized by a megaloblastic bone marrow there was no increase in the protoporphyrin content of the erythrocytes These observations are in accord with Stasney s direct observations on the protoporphyrin content of various types of bone marrow [11] His studies suggested that normoblasts contain protoporphyrin in considerable amount while megaloblasts do not

A high EP has not been found however in all conditions associated with a normoblastic bone marrow Thus in the bone marrow of patients L S (Banti s syndrome) W G (Banti s syndrome) and N A (congenital hemolytic jaundice)

42 32 and 40 per cent respectively of all of the nucleated cells were normoblasts Yet the EP values in the blood were 35 62 and 45 μg respectively Again in pyridoxine deficiency anemia in swine[11] [9] low values for EP have been found in the face of normoblastic marrow hyperplasia Watson[6] determined the EP in a typical case of thalassemia major and found it to be only 20 μg per 100 cc He speculated that this might be because protoporphyrin is not formed in appreciable amounts in the early erythroblast stage This could not be the explanation for the essentially normal EP values in our cases cited above since the normoblasts in the bone marrow were late forms Further studies of the EP content of various types and stages of red blood cells are needed

Theoretically it would seem that if hemoglobin synthesis is retarded due to factors other than a deficiency in protoporphyrin synthesis the amount of free protoporphyrin might be increased since protoporphyrin would not be the limiting factor This can be visualized as follows

(a) Porphyrin precursors → Protoporphyrin
(b) Protoporphyrin + Fe + Globin → Hemoglobin

If reaction (b) does not proceed and reaction (a) continues there would be an excess formation of protoporphyrin By the same reasoning if reaction (a) is the limiting factor it might be expected that the EP would be low or normal

As indicated earlier there is evidence that reticulocytes contain more free protoporphyrin than mature cells [10] It is not known whether this represents a small excess left over during hemoglobin synthesis or is merely a degradation product of hemoglobin It would seem more logical to assume that the presence of free protoporphyrin in reticulocytes represents uncompleted hemoglobin synthesis In the anemia of infection hemoglobin synthesis has been shown to be impaired An increase in EP under such circumstances could be readily understood In iron deficiency anemia lack of iron is the limiting factor If there is no defect in protoporphyrin synthesis one would expect an increase in EP Any other conditions which impair hemoglobin synthesis without interfering with protoporphyrin production should be characterized by increased EP—unless protoporphyrin is broken down or removed at a rate sufficient to prevent accumulation We have postulated that in pyridoxine deficiency protoporphyrin synthesis may be impaired [9] whereas in pernicious anemia protoporphyrin accumulation may be prevented by its conversion to bilirubin [30] thus explaining the low and normal EP values observed in these conditions respectively both of which are characterized by hyperferremia

In the last analysis however one must concur with Watson who pointed out[6] that until it is determined whether the protoporphyrin of reticulocytes is merely a small excess left over during hemoglobin synthesis or is purely a degradation product of hemoglobin and until it is known whether or not protoporphyrin is eliminated or built up into additional hemoglobin in the circulating erythrocyte its significance will remain uncertain

The significance of the plasma iron is clearer than that of free erythrocyte protoporphyrin Our observations are consistent with those described by

normal the plasma iron normal or high and the plasma copper usually normal Anemia due to iron deficiency and chronic infections was accompanied by an elevated EP, hypoferremia and hypercupremia In nephritis with anemia the EP was generally increased the plasma iron was low or normal and the plasma copper was increased Anemia associated with lymph node disorders or leukemia was accompanied by a normal or high EP a low or normal plasma iron and an increase in plasma copper In thalassemia minor the serum iron was normal hypercupremia was found in 4 out of 6 patients Thalassemia major was accompanied by both hyperferremia and hypercupremia Hyperferremia was present in all 4 patients with aplastic anemia who were studied In 2 patients with plumbism there was a marked increase in EP Hypocupremia was encountered only twice in one patient with severe nephritis and hypoalbuminemia and in one patient with hemochromatosis

TABLE 11 —*Summary of the Data*

Condition	Erythrocyte Protoporphyrin				Plasma Iron				Plasma Copper			
	No Pts	Low	Normal	High	No Pts	Low	Normal	High	No Pts	Low	Normal	High
Pernicious Anemia	17	1	16	0	28	1	16	11	12	0	9	3
Iron Deficiency	13	0	0	13	13	13	0	0	8	0	2	6
Chronic Infections	10	0	1	9	9	9	0	0	9	0	0	9
Nephritis	10	0	2	8	10	4	6	0	5	0	1	4
Lymphoma or leukemia	19	0	7	12	19	7	11	1	6	0	0	6
Aplastic Anemia	2	0	1	1	4	0	0	4	2	0	1	1
Thalassemia Major					5	0	1	4	3	0	0	3
Thalassemia Minor	1	0	1	0	6	0	6	0	6	0	2	4
Hemochromatosis	1	0	1	0	1	0	1	0	1	1	0	0
Plumbism	2	0	0	2	2	0	1	1	2	0	2	0
Myelophthisic Anemia	2	0	0	2	2	0	1	1				
Hemolytic Anemia	2	0	1	1	2	1	1	0	1	0	1	0

In general it was found that in conditions characterized by hypoferremia the EP and plasma copper were elevated (anemia of infection iron deficiency nephritis lymph node disorders and leukemia) Analyzing the data in another way it would seem that there was an increase in EP in anemic states associated with a normoblastic bone marrow due to a disturbance in hemoglobin synthesis i e iron deficiency anemia of infection nephritis lead poisoning and some cases of lymphoma and leukemia In contrast in pernicious anemia which is characterized by a megaloblastic bone marrow there was no increase in the protoporphyrin content of the erythrocytes These observations are in accord with Stasney s direct observations on the protoporphyrin content of various types of bone marrow [11] His studies suggested that normoblasts contain protoporphyrin in considerable amount while megaloblasts do not

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ACKNOWLEDGMENTS

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previous investigators cited already In conditions in which the amount of iron absorbed is decreased (inadequate dietary intake of iron etc) and in conditions in which the rate of elimination is increased (hemorrhage) the plasma iron is low In conditions in which the amount of iron going to the tissues is increased (anemia of infection) or in conditions in which hemoglobin is being rapidly synthesized (e g pernicious anemia during treatment) the plasma iron is low In conditions in which hemoglobin synthesis is reduced due to factors other than a lack of iron (pernicious anemia in relapse thalassemia major aplastic anemia pyridoxine deficiency in swine) the plasma iron is high In conditions in which hemoglobin catabolism is accelerated (hemolytic anemia) the plasma iron is high More than one of these factors may be operating as in hemolytic anemia where both hemo globin synthesis and hemoglobin destruction are accelerated In this event the plasma iron is dependent upon the balance of these factors and generally fluctuates depending upon which factor predominates at a given time

Knowledge of the absorption function and metabolism of copper especially in relation to erythropoiesis is so limited that interpretation of our findings is difficult if not impossible In general plasma copper is a more stable constituent of blood than is the EP or plasma iron An elevation of plasma copper has been a rather consistent finding in (1) the anemia of infection (2) lymphomas (3) leu kemia (4) iron deficiency (5) nephritis and (6) thalassemia No change was noted in pernicious anemia Low plasma copper values were infrequent We have observed hypocupremia only twice In the patient with osteomyelitis and hypercupremia who subsequently developed nephritis hypoalbuminemia and hypocupremia the last finding might be explained on the basis of the hypoalbuminemia if copper is bound to an albumin in the serum The finding of hypocupremia in the patient with hemochromatosis is interesting in view of Mallory s theory [8] that hemo chromatosis is due to copper poisoning The hypocupremia might indicate rapid mobilization of the copper into the tissues

Summary

1 A total of 108 erythrocyte protoporphyrin determinations has been made in 66 normal individuals The geometric mean ± standard error of the mean was 31 (26–38)

2 A total of 196 determinations of plasma iron in 92 normal individuals was made The mean ± standard error of the mean was 104 7 ± 3 4 μg per cent

3 In a total of 150 determinations of plasma copper in 105 normal individuals the mean ± standard error of the mean was 118 6 ± 1 2 μg per cent

4 No significant difference in plasma iron was noted between the sexes but in females the plasma copper was significantly higher and the erythrocyte proto-porphyrin slightly higher than in males

5 Erythrocyte protoporphyrin plasma iron and plasma copper determinations have been made in over 112 patients with a variety of clinical conditions associated with anemia In general it was found that in pernicious anemia in relapse the erythrocyte protoporphyrin values were normal the plasma iron normal or high and the plasma copper usually normal Anemia due to iron deficiency as well as the

anemia of infection were accompanied by high values for erythrocyte protoporphyrin hypoferremia and hypercupremia In nephritis with anemia the erythrocyte protoporphyrin was generally increased the plasma iron low or normal and the plasma copper increased Anemia associated with lymph node disorders or leukemia was accompanied by a normal or high EP a low or normal plasma iron and an increase in plasma copper Thalassemia major was found to be accompanied by both hypercupremia and hyperferremia in thalassemia minor the serum iron values were normal although hypercupremia was found Hyperferremia was noted in aplastic anemia In cases of plumbism the erythrocyte protoporphyrin was markedly increased Hypocupremia was noted only twice in one patient with severe nephritis and hypoalbuminemia and in one patient with hemochromatosis

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STUDIES ON HYPOPROTEINEMIA I HYPOPROTEINEMIA IN PATIENTS WITH GASTRIC CANCER ITS PERSISTENCE AFTER OPERATION IN THE PRESENCE OF BODY TISSUE REPLETION

By F Homburger M D and N F Young Ph D

With the technical assistance of Iris Forbes A B

Introduction

Hypoproteinemia occurs with greater frequency in patients with gastric cancer than in patients with other neoplasms or with benign lesions of the gastro intestinal tract This was noted earlier by various authors [1-8] and has been studied in this laboratory Hypoproteinemia becomes more pronounced and resistant to therapy following surgical treatment for cancer of the stomach than following similar operations for benign lesions of the stomach or for cancers of other organs

In previous studies from this laboratory it was shown[7] that the preoperative administration of large amounts of protein to patients with gastric cancer renders the postoperative hypoproteinemia less pronounced The present study revealed that hypoproteinemia once it is established in patients with gastric cancer following operation is persistent in spite of the administration of large amounts of protein adequate to result in significant increase of body tissue protein

Case Material and Methods

GENERAL PLAN OF STUDY

Patients who underwent partial gastrectomies for benign gastric ulcers and patients undergoing similar operations or exploratory laparotomies for cancer of the stomach were given high protein high caloric feedings as soon as possible following surgery Nitrogen balances were recorded for from 7 to 108 days and potassium calcium sodium and phosphorus balances were studied as well At regular intervals plasma volumes plasma protein concentrations and electrophoretic plasma protein fractions were determined It was thus possible to evaluate nitrogen retention body tissue repletion and plasma protein regeneration to compare the changes occurring in patients with benign gastric lesions to those observed in patients with gastric cancer and to evaluate the utilization and distribution of nitrogen in both groups

CASE MATERIAL

(Detailed case histories are given in the Appendix) The initial plasma protein values of all patients are recorded in table 1

Patients with Gastric Cancer

Three patients had cancer of the stomach which was operable and underwent partial gastrectomy (cases 4 5 and 6) These were 1 female and 2 males aged 60 46 and 50 respectively

Seven patients had inoperable cancer of the stomach Six of these underwent exploratory laparotomy

From the Laboratory of Clinical Investigation the Sloan Kettering Institute for Cancer Research New York

This study was aided by grants from the Teagle Foundation and the National Cancer Institute

TABLE 1

Case No	Days		Weight Kg			Plasma prot Gm /100 ml		Plasma volume		Total circulating protein		Nitrogen intake			Nitrogen output			Nitrogen retained		
	D*	PO†	Initial	End	Avg	Initial	End	Initial	End	Initial	End	Gm / day	Gm / day/ kg	Total Gm	Gm / day	Gm / day/ kg	Total Gm	Total Gm	Gm /day/ kg	% Intake
Gastric ulcers																				
								ml	ml	gm	gm									%
1	11	3	56 4	54 1	55 3	5 52	6 56	3290	2910	182 6	191 0	14 4	0 26	158	15 3	0 28	168	−10	−0 02	−6
2	10	3	56 8	58 6	57 7	4 85	6 66	3300	2540	160 1	169 2	28 5	0 49	285	22 4	0 39	224	61	0 11	31
3	10	3	47 7	46 4	47 5	5 62	5 66	2040	3300	114 6	186 8	—	—	—	—	—	—	—	—	—
Operable cancers																				
4	11	1	54 1	51 8	53 0	5 76	5 25	2220	2190	127 9	115 0	24 9	0 47	274	26 8	0 51	295	−21	−0 04	−8
5	12	2	75 0	—	—	6 13	6 15	3610	3060	221 3	188 2	—	—	—	—	—	—	—	—	—
6	18	[illegible]	66 4	—	—	5 24	6 56	4860	3520	254 7	230 9	—	—	—	—	—	—	—	—	—
Inoperable cancers																				
7	14‡	−3	63 2	63 6	63 4	6 13	6 81	2440	2380	149 6	162 1	35 3	0 56	353	15 7	0 25	157	196	0 31	56
8	7	0	47 7	50 9	49 3	6 47	5 06	2240	2300	144 9	116 4	40 0	0 81	280	22 6	0 46	158	122	0 35	44
9	14	−1	49 5	—	—	7 20	5 48	1820	1870	131 0	102 5	10 1	0 21	142	8 6	0 17	121	21	0 03	15
10	17	0	67 3	61 4	64 4	6 67	6 54	3490	4008	232 8	262 1	17 2	0 27	292	17 2	0 27	292	0	0	0
11	12	0	64 1	—	—	6 37	5 80	3450	3450	219 8	200 1	17 8	0 28	214	11 9	0 19	143	71	0 09	33
Inoperable cancers—long term studies																				
12	108	12	53 6	60 9	57 3	6 06	5 11	3100	3750	187 9	191 6	27 9	0 49	3017	24 2	0 42	2615	402	0 07	13
13	26	11	69 5	69 5	69 5	5 32	6 36	5160	4220	274 5	268 4	29 6	0 43	770	19 5	0 28	506	264	0 15	34

* Length of study days
† Beginning of study days after operation
‡ Nitrogen balance for 10 days only plasma protein change in 14 days

(cases 7 8 9 10 11 and 13) These were two females and four males aged 69 47 52 49 70 and 64 respectively

One patient (case 12) was found to have a resectable cancer of the stomach which was removed but firm large lymph nodes were felt in the portal region and in the omentum and were not removed This man was 65 years old

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Three patients with benign gastric ulcers were studied (cases 1 2 and 3) These were three males aged 51 45 and 53 This control group was kept small because previous work by Co Tui et al [8] had clearly shown that patients with gastrectomy for ulcers on a high nitrogen intake regenerate plasma protein within two weeks following operation and our findings confirmed this observation

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Time	Hydrolysate per feeding	Frequency (feeding)	Water to be added	Remarks	Parenteral 5% dextrose
	c	*h*			*cc*
Day of op					
6 hrs p o	—	—	30 cc q 1 hr	Alternate as required	
12 hrs p o	30	q 2	30 cc. q 1 hr	Same	
First day	Same	Same	Same		2500
Second day	Same	Same	Same		2500
Third day	30	q 1	30 cc q 1 hr	To rinse tube	1500
Fourth day	60	q 2	75 cc q 2 hrs	To rinse tube	1500
Fifth day	60	q 2	45 cc q 2 hrs 50 cc boiled milk added	After hydrolysate	1000

Sixth and seventh day as above Reduce parenteral fluid according to patient s ability to swallow small amounts of water by mouth Remove stomach tube on eighth day and start patient on routine seventh day gastrectomy diet

Methods

1 *Alimentation* Some of the patients had orojejunal feeding tubes inserted at operation and left in situ for as long as seven days postoperatively Some had external jejunostomy tubes for feeding purposes Unless otherwise specified casein hydrolysate Squibb was used as a source of nitrogen in all cases In general the plan included the administration of 0 6 Gm of nitrogen per day per Kg of body weight in a mixture of the hydrolysate in a 10 per cent dextrose solution with 60 Gm of Amphojel This feeding mixture was administered by the jejunal tube and/or by mouth and the total daily volume was adjusted to 720 ml A feeding schedule given in table 2 was followed as closely as possible After the first post operative period this regimen was supplemented by the usual gastrectomy hospital diet in amounts measured in the metabolic kitchen and analyzed in the laboratory for nitrogen content The caloric content of the total diet including parenteral alimentation was maintained at a level of approximately 1800 calories per day (except on the first and sometimes the second postoperative day when it was lower)

The hydrolysate was provided by the courtesy of the Squibb Co

TABLE 1

Case no	Days		Weight Kg			Plasma protein Gm/100 ml		Plasma volume		Total circulating protein		Nitrogen intake			Nitrogen output			Nitrogen retained		
	D*	PO†	Initial	End	Average	Initial	End	Initial	End	Initial	End	Gm/day	Gm/day/kg	Total Gm	Gm/day	Gm/day/kg	Total Gm	Total Gm	Gm/day/kg	Intake
								ml	ml	gm	gm									%
Gastric ulcers																				
1	11	3	56.4	54.1	55.3	5.52	6.56	3290	2910	182.6	191.0	14.4	0.26	158	15.3	0.28	168	−10	−0.02	−6
2	10	3	56.8	58.6	57.7	4.85	6.66	3300	2540	160.1	169.2	28.5	0.49	285	22.4	0.39	224	61	0.11	31
3	10	3	4[illegible].7	46.4	47.5	5.62	5.66	2040	3300	114.6	186.8	—	—	—	—	—	—	—	—	—
Operable cancers																				
4	11	1	54.1	51.8	53.0	5.76	5.25	2220	2190	127.9	115.0	24.9	0.47	274	26.8	0.51	295	−21	−0.04	−8
5	12	2	[illegible]5.0	—	—	6.13	6.15	3610	3060	221.3	188.2	—	—	—	—	—	—	—	—	—
6	18	2	66.4	—	—	5.24	6.56	4860	3520	254.7	230.9	—	—	—	—	—	—	—	—	—
Inoperable cancers																				
7	14‡	−3	63.2	63.6	63.4	6.13	6.81	2440	2390	149.6	162.1	35.3	0.56	353	15.7	0.25	157	196	0.31	56
8	7	0	47.7	50.9	49.3	6.47	5.06	2240	2300	144.9	116.4	40.0	0.81	280	22.6	0.46	158	122	0.35	44
9	14	−1	49.5	—	—	7.20	5.48	1820	1870	131.0	102.5	10.1	0.21	142	8.6	0.17	121	21	0.03	15
10	1[illegible]	0	67.3	61.4	64.4	6.67	6.54	3490	4008	232.8	261.1	17.2	0.27	292	17.2	0.27	292	0	0	0
11	12	0	64.1	—	—	6.37	5.80	3450	3450	219.8	200.1	17.8	0.28	214	11.9	0.19	143	71	0.09	33
Inoperable cancers—long term studies																				
12	108	12	53.6	60.9	57.3	6.06	5.11	3100	3750	187.9	191.6	27.9	0.49	3017	24.2	0.42	2615	402	0.07	13
13	26	11	69.5	69.5	69.5	5.32	6.36	5160	4220	274.5	268.4	29.6	0.43	770	19.5	0.28	506	264	0.15	34

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Plasma and blood transfusions were given in all cases on the day of operation and thereafter as sparingly as possible to avoid interference with the observations on protein regeneration Parenteral fluid intake was standardized as closely as possible 1000 ml of physiologic saline solution and 300 ml of dextrose in water on the second and only rarely on the third postoperative day All parenteral intake as well as other alimentation was carefully measured and recorded The patients received a daily intramuscular injection of a vitamin B complex preparation throughout the duration of the metabolic study Other medication was given as indicated The patients usually were allowed up on the second or third postoperative day but this was not standardized The 2 patients on whom long term metabolic studies were carried out for 26 and 108 days were placed on a constant basic diet which was the same every day

In addition to maintain the desired high nitrogen content these patients received part of their nitrogen intake in the form of protein hydrolysate (Casein Hydrolysate Squibb) and in the form of native protein (milk protein as Delcos Sharp and Dohme and Lactalbumin Squibb in unhydrolyzed form)

2 *Collection of specimens* Urines were collected in 24 hour specimens preserved at pH 3 with acetic acid and kept in the refrigerator without preservative Vomitus and gastric aspiration fluid were collected in 24 hour samples and kept in the refrigerator without preservative

3 *Methods of Analysis*

Urine Urinary nitrogen was measured by a micro Kjeldahl method Potassium and sodium were measured by flame photometry[9] phosphorus was determined by Fiske and Subbarow s method [10] and calcium by the method of Schohl and Pedley [11] Creatinine determinations[12] were u ed to evaluate the accuracy of the collections

Stools A commercial homogenizer was used for the thorough mixing of stools which were then made up to a standard volume by the addition of distilled water Nitrogen was measured by a micro Kjeldahl method and minerals were determined in dry ashed aliquots by the same methods used in the urine

Blood Plasma protein was measured by a micro Kjeldahl method and corrected for nonprotein nitrogen The plasma protein components were estimated by electrophoresis by Dr M L Petermann [13] Plasma volumes were measured by the use of Evans blue [14]

RESULTS

Body Tissue Repletion Table 1

Repletion within one month after operation In only 2 of the 3 *ulcer* cases studied were nitrogen balance studies done The third case is included because while nitrogen output was not measured the nitrogen intake was known and was at least as high as in the two others As previously shown by Co Tui et al [8] and by Reigel [15] positive nitrogen balance† was obtained in both cases the usual postoperative nitrogen loss therefore was offset by adequate nitrogen utilization This was achieved on intakes of 0 26 and 0 49 Gm of nitrogen per Kg per day or considerably less than had been planned originally All patients with gastric cancer were in nitrogen balance or had positive nitrogen balance 7 to 20 days following operation There was essentially no difference between those having undergone exploratory laparotomy and the one case with gastric resection in which nitrogen balance studies had been done (case 4) The least marked nitrogen retention actually occurred in the latter case The nitrogen intake in the cases with cancer ranged from 0 2 Gm per Kg per day to 0 81 Gm per Kg per day with an average of 0 43 Gm per Kg

Beta Symplex was u ed (Winth op) which has the following composition thiamin 10 mg riboflavin 5 mg calcium pentothenate 5 mg niac namide 50 mg

† A patient is considered to be in nitrogen balance when the loss of nitrogen exceeds the intake by less than 0 01 Gm per Kg per day

per day An average of 26 per cent of the ingested nitrogen was retained as compared to 12 per cent (31 and −6 per cent) in the ulcer group

Repletion more than one month after operation Studies of nitrogen and mineral balances were made in 2 cases of gastric cancer following exploratory laparotomy (case 12) and following palliative gastrectomy (case 13) during periods of 26 and 108 days and starting 12 and 11 days after operation These showed marked nitrogen retention 402 Gm or 0 065 Gm per kg per day for 108 days equal to 13 per cent of the intake and 264 Gm or 0 15 Gm per kg per day or 34 per cent of the intake

TABLE 3—*Comparison between Theoretic and Actual Nitrogen Intake and Changes of Circulating Protein*

Case	Type of case	Theoretic nitrogen requirement	Nitrogen actually received	Protein observed change	Protein calculated change†	Difference
				gm	gm	gm
1	Ulcer	323	158	+9 3	−2 1	+11 4
2	Ulcer	395	285	+9 1	+12 7	−3 6
3	Ulcer	261	—	+[illegible]1 7	—	—
4	Operable cancer	241	274	−13 0	−4 3	−8 7
5	Operable cancer	468	—	−33 0	—	—
6	Operable cancer	473	—	−23 [illegible]	—	—
7	Inop cancer	258	353	+13 6	+41 0	−27 4
8	Inop cancer	163	280	−28 5	+25 4	−53 9
9	Inop cancer	246	14	−27 6	+4 4	−32 0
10	Inop cancer	205	292	+29 3	0	+29 3
11	Inop cancer	262	214	−19 9	+14 8	−44 7
12	Inop cancer long term study	654	3017	−1 0	+83 0	−84 0
13	Inop cancer long term study	564	770	−7 1	+55 0	−62 1

Theoretic Nitrogen Intake means the amounts of nitrogen required for correction of the plasma albumin deficit (Assumed normal albumin 4 6 Gm /100 ml All calculations are based on values obtained by the Howe method because this method was the one used in evaluating the proportion of albumin to the remaining body protein ref Elman R Protein deficiency in surgical patients and its correction J Am Dietet A *18* 141–144 1942. The total calculated nitrogen requirement includes 4 Gm for daily maintenance during the period of study the tissue protein loss calculated from the albumin lost and assumes a loss of 50 per cent of the ingested nitrogen (above the maintenance requirement of 4 Gm per day))

† The calculated change of plasma protein is based on the amounts of nitrogen retained or lost assuming that plasma protein represents one thirtieth of the total body protein

in 26 days There was concomitant retention of phosphorus and potassium in the proportions in which these minerals are known to exist in protoplasm In these two instances the ability of these patients to build body tissue is thus well demonstrated

Plasma Protein Regeneration Table 3

Regeneration within one month after operation In all patients with gastrectomy for benign ulcers an increase in circulating plasma protein occurred promptly The extent of this increase varied widely in these 3 patients and was predominantly in

Plasma and blood transfusions were given in all cases on the day of operation and thereafter as sparingly as possible to avoid interference with the observations on protein regeneration Parenteral fluid intake was standardized as closely as possible 1000 ml of physiologic saline solution and 300 ml of dextrose in water on the second and only rarely on the third postoperative day All parenteral intake as well as other alimentation was carefully measured and recorded The patients received a daily intramuscular injection of a vitamin B complex preparation throughout the duration of the metabolic study Other medication was given as indicated The patients usually were allowed up on the second or third postoperative day but this was not standardized The 2 patients on whom long term metabolic studies were carried out for 26 and 108 days were placed on a constant basic diet which was the same every day

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calculated figures is much wider in the group of cancer patients than in the two ulcer cases and that it becomes extreme in the long term studies

Discussion

Hypoproteinemia continues in gastric cancer patients after operation in spite of adequate body tissue repletion

Causes of hypoproteinemia are (1) inadequate formation (2) increased utilization or destruction or (3) abnormal distribution of circulating proteins Inadequate formation may be due to (a) insufficient protein intake (b) excessive nitrogen loss or (c) specific defect in serum protein synthesis In patients with gastric cancer who show hypoproteinemia before operation insufficient intake and excessive loss of nitrogen have been eliminated by earlier studies [4,5] The present investigation supplies evidence that inadequate intake and excessive loss of protein are not responsible for the persistence of the hypoproteinemia in gastric cancer patients after operation

One concludes that either inadequate fabrication or distribution is responsible for *hypoproteinemia in gastric cancer The defect is probably nonspecific as similar* observations have been made in patients with tuberculosis [18] It may be related to the gastro-intestinal tract to the liver or to the adrenal cortex

Further studies are in progress to determine if the hypoproteinemia is due to decreased synthesis to increased utilization or destruction or to abnormal distribution of proteins

Appendix

Summaries of Clinical Histories of the Patients Studied

CASE 1

(B J 81337 11) colored male of 59 years Admitted 6-25-46 discharged ~-9-46

History Anorexia for 2 years Pain in midepigastrium for 2 months before admission 13 lbs weight loss in 2 years No vomiting no tarry stools

Physical examination Tenderness in epigastrium on deep pressure no other findings

X-ray examination Gastric ulcer lesser curvature

*Laboratory data** Hemoglobin 82% RBC 4 800 000 WBC 6 100 normal differential count Urine negative Serum chlorides 95 105 meq /l Serum bilirubin 1 0-1 4 mg /100 ml Blood urea nitrogen 12 2-30 4 mg 100 ml

Gastric analysis† Free HCl 0 0 22 24 15
Total HCl 12 18 35 36 30

Operation Partial gastrectomy 6-24-46

Pathology Chronic peptic ulcer

Course Uneventful postoperative recovery Discharged 15 days postoperatively asymptomatic since

CASE 2

(M P 81622 42) white male of 45 years Admitted 5-29-46 discharged 6 5-46

History Peptic ulcer pain 7 years ago successfully treated by dietary measures Recurrence one year ago unrelieved by diet In the 4 months before admission frequent vomiting Only 4-5 lbs weight loss

* Data included in the paper are not repeated in the appendix

† First sample before 0 5 mg of histamine following samples every 15 minutes thereafter Acid in ml of N/10 NaOH

the globulin fraction in one The increase of the circulating plasma protein was of the same order of magnitude as that observed by Co Tui under similar conditions and not to be ascribed to changes of plasma volume alone (table 1) In all patients with gastric cancer except two (case 10 and case 7) there was a decrease of circu lating plasma protein during the period of study In case 10 the increase observed follows a rise of the plasma volume more marked than in most of the other cases and thus may be only illusory In case 7 there was an actual increase entirely caused by a rise of plasma globulin

TABLE 4 —*Changes in Globulin and Albumin as Determined by Electrophoresis and Plasma Volume Measure ment (Initial Values at Beginning of Study Final Value at End of Period—Stated in Last Column)*

Case no	Type case	Cha ge f pla ma volume	Album n†			Gl b l n†			Days
			In tial	Final	Cha ge	In tial	Fin l	Cha ge	
		%			%			%	
1	Benign ulcer	−11 5	80 0	81 5	+2 0	101 5	100 4	+7 9	11
2	Benign ulcer	−23 0	69 5	78 9	+13 5	90 6	90 3	−0 3	10
3	Benign ulcer	+6 2	46 4	60 0	+29 3	68 0	126 5	+86 0	10
4	Operable cancer	−1 4	57 2	43 6	−23 8	70 6	71 3	+0 4	11
5	Operable cancer	−15 5	74 7	57 5	−23 0	146 6	130 6	−10 9	12
6	Operable cancer	−27 6	106 0	91 9	−13 3	149 0	139 0	−6 7	18
7	Inoperable cancer	−2 7	70 2	64 5	−8 1	79 3	97 5	+23 3	10
8	Inoperable cancer	+2 7	*						7
9	Inoperable cancer	+2 7	53 3	37 4	−28 1	74 0	62 3	−15 8	14
10	Inoperable cancer	+14 6	86 5	101 8	+17 7	146 2	160 3	+10 0	17
11	Inoperable cancer	0	83 1	65 2	−21 7	136 7	134 9	−1 3	12
12	Inoperable cancer—long term study	+21 0	81 9	90 8	+10 9	106 0	101 0	−4 7	108
13	Inoperable cancer—long term study	−18 4	121 5	119 0	−2 85	155 0	149 5	−3 6	26

* Only total protein was determined

† Refers to total circulating fractions

Regeneration more than one month after operation The changes of circulating plasma protein in these two cases following 26 and 108 days of postoperative high protein feeding were insignificant and probably within the range of technical errors

Comparison of Theoretic and Actual Plasma Protein Increases Table 4

Studies by Weech[16] in dogs showed that the plasma albumin represents $\frac{1}{30}$ of the body protein and by transferring this assumption to man one can estimate the amounts of circulating albumin which theoretically should be formed from a given amount of retained food protein Applying these relationships Elman[17] has de vised a calculation to evaluate the nitrogen need of a depleted individual from his plasma albumin concentration The further assumption may be made that albumin represents most of the plasma proteins lost in protein depletion and that thus the total protein may be substituted for albumin in such cases These rough theoretic figures demonstrate that on the whole the discrepancy between the actual and the

Urine negative except for occasional 1+ albumin Serum chlorides 97–103 meq /l Serum bilirubin 0 6 4 8 mg /100 ml Serum cholesterol total 141 free 52 esters 89 mg /100 ml Fasting blood sugar 99 mg /100 ml Blood urea nitrogen 11 3–25 mg /100 ml

Gastric analysis Free HCl = 0
Total HCl 26 10 – 10 15

Operation No metastasis seen in liver lymph nodes or peritoneum A large tumor involving the greater gastric curvature and extending into the transverse colon and the omentum was found Subtotal gastrectomy and resection of the medial portion of the transverse colon and a large portion of the omentum was performed A Mikulicz colostomy was done (– 19–46) On 8 8 46 patient developed acute intestinal obstruction and a number of fibrous adhesions were dissected at an emergency laparotomy The patient recovered and was discharged On 10–4 46 colostomy was closed

Pathology Adenocarcinoma Grade III invading entire thickness of gastric wall and extending into mucosa of colon

Course Patient now in good health

CASE 6

(M S 82126-53) white male of 50 years Admitted 6–30–46 discharged 7–22 46

History Midepigastric pain for 7 months with 12 lbs weight loss

Physical examination Negative

X-ray examination Carcinoma of lesser curvature near cardia

Laboratory data Hemoglobin 48–87% RBC 2 300 000–3 000 000 WBC 3 700–9 600 normal differential count Urine negative Serum chlorides 98–108 meq /l Blood urea nitrogen 12 1–36 8 mg /100 ml

Gastric analysis Free HCl 10 25 30 55 60
Total HCl 20 40 38 75 80

Operation No metastasis seen in liver lymph nodes or peritoneum Subtotal gastrectomy

Pathology Adenocarcinoma of stomach

Course Uneventful recovery

CASE 7

(B J 809 S 8) white male of 69 years Admitted 3–22 46 discharged 4–8 46

History Weight loss of 6 lbs in 3 months and loss of appetite

Physical examination Negative

X-ray examination Large mass in pars media of stomach

Laboratory data Hemoglobin 73–85% RBC 3 700 000–4 600 000 WBC 6 100–10 200 Normal differential count Urine negative Serum chlorides 100–108 meq /l Serum bilirubin 0 7 mg /10 ml Serum cholesterol total 29 free 61 esters 136 mg 100 ml Fasting blood sugar 88 mg 100 ml Blood urea nitrogen 12 4–13 2 mg / 100 ml

Gastric analysis Free HCl 0 0 qns qns 0
Total HCl 24 – qns qns 8

Insufficient quantity

Operation Laparotomy 3 28–46 A large mass was found involving the stomach extending along both curvatures Numerous metastatic nodules in liver gall bladder and nodes along aorta Inoperable case

Pathology Metastatic adenocarcinoma

Course Complicated by hypoproteinemia (hypochloremia and edema) Patient was discharged no follow up notes

CASE 8

(G Y 80364 96) white male of 47 years Admitted 1–27–46 discharged 2–17–46

History Past history of glycosuria Negative urine at time of admission Weight loss of 60 lbs in 18 months Epigastric fullness and anorexia for 12 months

Physical examination Negative

X ray examination Duodenal ulcer

Laboratory data Hemoglobin 89 103% RBC 4 100 000–4 600 000 WBC 4 000–8 300 normal differential count Urine negative Serum chlorides 94–102 meq /l Serum bilirubin 1 1 mg /100 ml Fasting blood sugar 89 mg /100 ml Blood urea nitrogen 16 3 26 4 mg /100 ml

Gastric analysis Free HCl 25 40 60 55 50

Total HCl 44 48 64 66 60

Operation Partial gastrectomy 5–22–46

Pathology Gastric ulcer scars in duodenum fibrous adhesions

Course Uneventful recovery Asymptomatic in April 1947

CASE 3

(F H 82072 17) white male of 53 years Admitted 6–25–46 discharged 7–17–46

History From 18 months to 1 year before admission pain after eating Weight loss in that period 25 lbs Vomiting for about 4 weeks

Physical examination Negative

X-ray examination Crater of ¾ inches in lesser curvature probably benign ulcer

Laboratory data Hemoglobin 81% RBC 3 ,00 000–4 500 000 WBC 5 000–15 000 Urine negative Serum chlorides 96–102 meq /l Serum bilirubin 0 7–1 3 mg /100 ml Fasting blood sugar 87 mg /100 ml Blood urea nitrogen 11 9–20 mg /100 ml

Gastric analysis Free HCl 55 32 28 31 35

Total HCl 28 50 65 –8 58

Operation Partial gastrectomy 7 2–46

Pathology Chronic peptic ulcer (gastric)

Course Patchy pulmonary infiltration on eighth postoperative day with moderate elevation of temperature Responded well to penicillin Asymptomatic since then

CASE 4

(K H 82018 34) white female of 60 years Admitted 6–27–46 discharged 7–19–46

History Abdominal pain of 2 months duration and weight loss of 20 lbs in 4 months before admission

Physical examination B P 160/85 soft systolic aortic murmur Rales throughout chest emphysematous thorax Mass and tenderness in left upper quadrant Difficult examination because of failure to relax abdominal wall

X ray examination Advanced gastric carcinoma of the body of the stomach and the pyloric region

Laboratory data Hemoglobin 63–88% RBC 3 800 000–4 200 000 WBC 4 400 16 800 Urine negative Serum chlorides 93–106 meq /l Serum bilirubin 0 6–1 2 mg / 100 ml

Gastric analysis Free HCl = 0

Total HCl 5 4 4 6 6

Operation Subtotal gastrectomy (4/5) Hoffmeister anastomosis 7–1–46 No liver metastasis or peritoneal implants found

Pathology Diffuse gelatinous adenocarcinoma extension to the fat about nodes Lymph nodes proper were clear

Course Uneventful recovery Patient has left town no follow up record d

CASE 5

(S S 82300-55) white male of 46 years Admitted 7–15–46 discharged 8–24–46 Readmitted for closure of colostomy 9–30–46 discharged 10–14–46

History 3 months persistent epigastric pain following fall from horse Lost 60 lbs in 4 months and noticed progressive weakness

Physical examination Essentially negative

X-ray examination No gastro-intestinal examination

Laboratory data Hemoglobin 64–91% RBC normal WBC 9 000–15 000 normal differential count

Operation Laparotomy 6–28 46 Extensive carcinoma with metastasis to nodes and liver
Pathology Adenocarcinoma Grade III
Course Convalescence complicated by bronchial pneumonia

CASE 12

(K R 83598 116) white male of 65 years Admitted 11–10–46 discharged 6–2–4,
History Weight loss during one year preceding operation of about 30 lbs Several bouts of dark black stools and rectal bleeding
Physical examination Negative
X-ray examination Filling defect involving almost the entire pyloric portion
Laboratory data Hemoglobin 8 4–54% RBC 1 190 000–5 000 000 WBC 4 700–8 800 normal differential count Urines negative Serum chlorides 92–104 meq /l Serum bilirubin 0 62 mg /10 ml Thymol turbidity 1 85 ml Cephalin flocculation 24 hours negative Hippuric acid excretion 1 5 Gm Bromsulfalein retained in blood 30 minutes 2% 45 minutes 2% Serum cholesterol total 104 free 36 esters 68 mg /100 ml Blood urea nitrogen 16 3–22 mg /100 ml
Gastric analysis Free HCl = 0
Total HCl 20 22 28 50 34
Operation On 11–25–46 a large bulky lesion was found in the greater curvature of the stomach a few nodes were palpated in the gastro-hepatic ligament The tumor was removed and a gastro-jejunostomy was done Liver and peritoneum free of metastases
Pathology Gelatinous adenocarcinoma Grade III extensive lymphatic permeation metastases to nodes
Course Essentially uneventful postoperative course This study was continued up to the 205th post operative day [19 b] The patient died of carcinomatosis 66 weeks after operation Reviews of additional articles bearing on this subject which have appeared since this paper was submitted are noted in the references [1 b]

CASE 13

(S J 109237 4) white male of 64 years Admitted 8–28 46 discharged 10–14–46
History Weight loss of 40 lbs in 4 months before admission Hematemesis one month before admission epigastric fullness
Physical examination Mass in epigastrium
X-ray examination Polypoid carcinoma of stomach (fundus and body)
Laboratory data Hemoglobin 46–76% RBC 2 200 000–3 400 000 Hematocrit 18–37% WBC 4 500–10 600 normal differential count Normal urine Serum chlorides 108 meq /l Serum bilirubin 1 2 mg /100 ml Fasting blood sugar 107 mg /100 ml Blood urea nitrogen 15 9 mg /100 ml
Gastric analysis Free HCl = 0
Total HCl qns 14 14 12 10
Operation 9–9–46 Large tumor from cardia through antrum among lesser curvature Liver studded with metastases Exploratory laparotomy biopsy from liver metastases
Pathology Gelatinous adenocarcinoma
Course Died Dec 1946

SUMMARY AND CONCLUSIONS

1 The existence of hypoproteinemia in patients with gastric cancer has once again been observed

2 The intractability of this type of hypoproteinemia in the postoperative phase to treatment with high protein diets and in the presence of positive nitrogen balance has been demonstrated

3 Long term studies in two patients for 28 and 108 days respectively suggest that the persistence of hypoproteinemia in patients with gastric cancer in positive

Physical examination Evidence of weight loss large abdominal mass

X ray examination Large mass in region of cardia and larger curvature

Laboratory data Hemoglobin 58–95% RBC 3 000 000–4 600 000 WBC 5 000–9 400 Normal urine Serum chlorides 101–105 meq /l Serum bilirubin 0 5–0 8 mg /100 ml Serum cholesterol total 121–250 free 42–71 esters 79–179 mg /100 ml Blood urea nitrogen 12 0–28 4 mg /100 ml

Gastric analysis Free HCl = 0

Total HCl 20 10 12 8 10

Operation Laparotomy 2–5–46 Large tumor mass involving gastric cardia and greater portion of fundus as well as mesentery spleen pancreas and lymph nodes Inoperable External jejunostomy

Pathology No biopsy material taken

Course Patient died 3–26–46 No necropsy

CASE 9

(B L 80901 6) white female of 52 years Admitted 3–19–46 discharged 4–9–46

History Dull abdominal pain for 4 months small weight loss

Physical examination Negative

X-ray examination Polypoid infiltrating cancer of distal segment of stomach

Laboratory data Hemoglobin 65–82% RBC 3 200 000–3 500 000 WBC 7 200–7 600 normal differential count Urine 4+ sugar acetone 1+ (probably after glucose infusion All subsequent urines negative) Serum chlorides 91–103 meq /l Serum bilirubin 0 7 mg /100 ml Fasting blood sugar 91–103 mg /100 ml Blood urea nitrogen 8 5–20 mg /100 ml No gastric analysis reported

Operation Laparotomy and external jejunostomy 3–26–46 Large mass involving stomach extension into lesser and greater omenta multiple metastases in liver (biopsy material taken)

Pathology Metastatic adenocarcinoma

Course No follow up notes

CASE 10

(S S 81726-51) white male of 49 years Admitted 5–28–46 discharged 6–26–46

History Weakness of 3 months duration and postprandial epigastric pain and tarry stools of one month duration No evidence of weight los

Physical examinati n Abdominal mass 8 cm in diameter

X ray examination Carcinoma of antrum of stomach

Laboratory data Hemoglobin 51–81% RBC 2 500 000–3 800 000 WBC 5 300–14 000 normal differential count Urine negative Serum chlorides 95–104 meq /l Serum bilirubin 0 6 mg /100 ml Serum cholesterol total 141 free 52 esters 89 mg /100 ml

Gastric analysis Free HCl = 0

Total HCl 15 8 10 12 12

Operation Laparotomy and exclusion gastroenterostomy 6–7–46 Large tumor of gastric antrum adherent to pancreas and meso sigmoid and colic vessels Numerous metastases in liver Firm lymph nodes

Pathology None reported

Course No follow up notes

CASE 11

(G G 82040-21) white female of 70 years Admitted 6–23–46 discharged 7–20–46

History Nocturia due to cystocele Marked weight loss (70 lbs) in 5 months before admission Anorexia and heart burn for one year prior to admission Negative gastro intestinal x ray examination some months before admission

Physical examination Mass of 10 cm diam ter in mid abdomen

X ray examinati n Gastric carcinoma

Laboratory data Hemoglobin 61–94% RBC 3 700 000–4 400 000 WBC 5 500–11 300 normal differential count Urine essentially negati e Serum chlorid s 93 102 m q l Serum bilirubin 1 5–2 6 mg /100 ml Blood urea nitrogen 12 8–36 2 mg 100 ml

Gastric analysis Free HCl = 0

Total HCl 20 10 qns 1 16

STUDIES OF PHOSPHORUS METABOLISM IN MAN II A STUDY OF THE PERMEABILITY OF THE HUMAN ERYTHROCYTE TO INORGANIC PHOSPHATE IN VITRO BY THE USE OF RADIOACTIVE PHOSPHATE (P^{32})

By F H L Taylor Ph D S M Levenson M D and M A Adams B A

With the technical assistance of Mary Kendrick B S

IT IS A distinct pleasure to dedicate the first published* of a series of articles from this laboratory on the use of radio isotopes in human physiologic studies to Dr George R Minot It was under his direction that our venture into this field was undertaken

Radio phosphorus has been used in the examination of various phases of phosphorus metabolism and the results have been summarized by Hevesy[1] and Kamen[2] Most of the data presented in the literature were obtained in animals and by use of dosages of isotopic P^{32} in excess of what may be considered tracer amounts Human studies on the metabolism of P^{32} in tracer amounts are few in number most of the interest of previous authors having been directed to the therapeutic uses of the isotope Since normal metabolic processes may be deranged by larger than tracer doses of P^{32} it seemed of value to investigate the metabolism of phosphorus in the human body using P^{32} as a tracer in doses low enough to cause no demonstrable metabolic disturbance These studies will be presented elsewhere[4] The use of P^{32} in such concentrations immediately imposes problems of revisions of methods and further considerations of accuracy of counting technics These will be described fully in another communication[5]

The first consideration in the attack on phosphorus metabolism was the resolution of the controversy of phosphorus exchange between cells and plasma of the circulating blood The present communication is concerned with the distribution of phosphorus between red cells and plasma in in vitro studies of the phosphorus exchange between these two media Eisenmann et al [6] have shown in an in vitro system to which inorganic phosphate together with P^{32} were added to human whole blood that at 38 C phosphate entered the red cells freely while at 7 C the exchange was minimal In their experiments 5 to 101 mg phosphorus were added to 100 ml of whole bood They also stated that under their experimental conditions radio phosphorus accumulated in the cells out of proportion to the amount of inorganic phosphate present Their observations were made on whole blood and plasma and the cell concentrations of phosphorus compounds were calculated using hematocrits

In the present communication no phosphate was added except that represented by the P^{32} which was negligible since the specific activity of the isotopic sodium

From the Thorndike Memorial Laboratory Second and Fourth Medical Services Harvard Boston City Hospital and the Department of Medicine Harvard Medical School Boston

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* No I of this series is in preparation

nitrogen balance is probably not due to a marked degree of depletion of tissue protein stores alone These patients retained more protein than would have been necessary to replenish depleted tissues

4 It seems more likely that while such patients are capable of tissue protein synthesis they fail to shift new protein into the blood stream

ACKNOWLEDGMENT

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Results

Distribution of Phosphorus Fractions between Plasma and Erythrocytes

The data are presented in table 1 It will be observed that the distribution between red blood cells and plasma on the initial nonincubated blood were in the range of those normally accepted After four hours incubation at 37 5 C (experiments 1 2 3) there was no net exchange between cells and plasma of any of the phosphate compounds beyond experimental errors of the methods employed There was a slight increase of the plasma content of all of the forms of phosphorus studied as incubation proceeded The hematocrit increased slightly or remained unchanged during the experiment Minimal hemolysis was present in all samples

TABLE 1 —*D stribut on of Pho phorous Compounds bet een Plasma and Erythrocytes*

Exp	Type	Temp °C	Hct	Initial (5 minutes) Total P mg per 100 ml		Inorg P mg per 100 ml		Total acid soluble P mg per 100 ml		After 4 hours incubation Hct	Total P mg per 100 ml		Inorg P mg per 100 ml		Total acid soluble P mg per 100 ml	
1	Plasma	37 5		12 8	(7 4)	3 0	(1 7)	3 2	(1 8)		14 7	(9 4)	4 4	(2 3)	4	(2)
	Red blood cells	37 5	41 8	63 4	(26 5)	7 5	3 1)	38 8	(16 0)	43 0	51 2	(22 0)	8	(3 3)	34 6	(14 9)
2	Plasma	37 5		13 1	(7 4)	3 0	1 7)	3 2	(1 7)		15 3	(8 7)	4 0	(2 3)	4 5	(2 5)
	Red blood cells	3 5	43 2	57 5	(24 8)	6 3	(2 7)	39 8	(16 8)	43 3	5 5	(25 1)	6 3	(2 8)	39 8	(16 3)
3	Plasma	37 5		11 6	6 3)	2 9	(1 6)	2 8	(1 5)		15 2	(9 3)	5 0	(2 7)	5 6	(3 1)
	Red blood cells	37 5	45 3	55 2	(25 0)	7 3	(3 3)	31 4	(14 3)	45 5	47 6	(21 7)	7 7	(3 5)	33 2	(15 2)
4	Plasma	7		8 7	5 0)	2 2	(1 3)	2 3	(1 3)		9 3	(5 4)	2 1	(1 2)	2 2	1 3)
	Red blood cells	7	41 4	57 8	(24 5)	8 5	(3 5)	38 7	(16 4)	41 9	58 6	(24 6)	8 1	(3 4)	4 2	(18 9)

Hct —Hematocrit [illegible]

[illegible] mg per 100 ml of cell or plasma figures in parentheses [illegible] values calculated for whole blood distribution [illegible] hematocrit

At 7 C no change in hematocrit or distribution of phosphorus compounds occurred nor was hemolysis of red blood cells observed

There was no indication that inorganic phosphate of the red blood cells increased at the expense of the organic fraction during four hours of incubation at 37 5 C

Acid soluble phosphorus in plasma was accounted for within the limits of error by the inorganic phosphorus both in the initial bloods and after the four hour incubation period at either 7 C or 37 5 C

The data of table 1 include in parentheses a recalculation of the various phosphorus constituents as distributed in whole blood using the observed hematocrit and known dilutions of the blood as the basis of calculation

When incubation was continued for twenty three hours at 37 5 C a marked in

phosphate used was very high The in vitro studies presented in this paper have been extended to similar studies in vivo in man which will be presented elsewhere [4]

Methods

Blood from normal human subjects was collected in bottles containing dried potassium ammonium oxalate solution which was prepared by dissolving 3 Gm of ammonium oxalate and 2 Gm of potassium oxalate by dissolving in distilled water to a volume of 100 ml For each ml of blood 0 04 ml of the anti coagulant was used Such an oxalate mixture caused no change in cell size P^{32} in the form of sodium phosphate solution was added to the whole blood the volumes of the radio active phosphate solution varying from 0 58 to 2 08 ml per 100 ml of whole blood with activities of P^{3} varying between 900 and 12 000 counts per second as regi tered on our counter Samples of the radioactive phosphate solution added were utilized as standards for each experiment and were counted at intervals throughout the exp ri ments to correct for the decay of P^{32} Counting was performed with a Geiger Mueller tub with fixed geometry and the totalizing was accomplished by the use of an autoscaler † The background count in this laboratory averaged 0 23 counts per second In none of these exp rim nts was there any count lower than ten times the background In all exp rim nts samples were removed for the determination of the hematocrit about five minutes after the addition of P^{32} to the blood In this same initial sample chemical deter minations of total total acid soluble and inorganic pho phorus were mad in both plasma and red blood corpuscles In two of the experiments the P^{32} content of the total phosphorus of plasma and cells was also measured in the initial blood sample

The blood was then allowed to stand in contact with P^{3} with frequent gentle shaking for a p riod of four hours In three cases this incubation took place at a temp rature of 37 5 C and in the fourth in tance the blood was kept for four hours at 7 C At the end of four hours the hematocrit was again d termined total total acid soluble and inorganic phosphorus were determined chemically in plasma and red blood cells and the P^{32} concentration was measured in all these phosphate fractions

Blood samples were centrifuged for ten minutes at 2000 rpm to separate the plasma from the red blood corpuscles The plasma was dra vn off as completely as possible and the blood cells were washed once with cold 0 9 per cent sodium chloride solution The washed cells were centrifuged and the sup rnatant saline drawn off and discarded The red blood corpuscles were then frozen at −20 C in order to produce hemolysis and to prevent any hydrolysis of organic phosphorus complexes For analy is the frozen cells were thawed and diluted with the amount of distilled water required for each determination The exact technics employed are described elsewhere [5]

The total phosphorus of both plasma and red blood cells was determined by the colorimetric molyb date method of Fiske and Subbarow[7] preceded by digestion with sulfuric and nitric acid Total acid soluble and inorganic phosphorus vere determined by Fiske and Subbarow s method in trichloracetic acid filtrates prepared from plasma and red blood corpuscles

To determine the total amount of radioactive pho phorus present in plasma and cells samples were simply dried and read with the Geiger Mueller tube and autoscaler The size of the samples as de pendent on the amount of P^{2} originally added to the blood The radioactivity of the total acid soluble fraction of both plasma and red blood corpuscles as measured in dried samples of the trichloracetic acid filtrates [5] Inorganic phosphorus vas determined by precipitation with calcium chloride as described by Fiske and Subbarow [8] The precipitated calcium phosphate as redissolved in acid and samples taken from this solution vere dried in order to measure the concentration of radioacti e pho phorus in the inorganic phosphorus fractic n Certain corrections for geometry film thickness and protein volume ere made and applied to all estimations of P^{3} It was estimated that the methods u ed were accurate to plus or minus 5 per cent to 10 per cent

The results are expressed in one of several ways either as the percentage of the amount of P^{2} added to the reaction flask or as spe ifi activities obtained by di iding the p r cent of the added amount of P^{32} by the milligrams of the gi en pho phorus compound per oo ml of plasma cell or whole bloods The use of the percentage of added P^{3} rather than the n mber of counts as the basis of calculation p rmitted direct comparison of the different experiments despite v ri tion in the actu l amount of P^{3} added

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P^{32} , as supplied by Monsanto Chemical Comp ny Clinton L borato ies Oak Ridge Tennessee

† Tracerlab Boston Massachusetts

Most of the P^{32} was in the inorganic fraction of the acid soluble phosphate From 6 to 14 per cent of the added P^{32} was found in the organic fraction of the acid soluble phosphorus There was no indication that in four hours there was any exchange to the nonacid soluble organic fractions At 7 C essentially all of the P^{32} was in the inorganic form

From a study of the specific activities presented in table 2 it will be seen that the specific activities of the inorganic phosphate of the cells equals or exceeds that of the inorganic fraction in the plasma at the end of four hours

The ratio of the P^{32} activity, in terms of percentage of the isotope added between the red blood cell and plasma $\left(\frac{\text{per cent added } P^{32} \text{ in R B C}}{\text{per cent added } P^{32} \text{ in plasma}}\right)$ ranged between 1 4 and 1 7 with a mean of 1 5 In in vivo studies on five normal men five hours after injection of 100–200 microcuries of P^{32} this ratio was 6 4 In these instances the plasma level of P^{32} was rapidly falling for obvious physiologic reasons which were not duplicated in the in vitro experiments

Discussion

The data presented amply confirm the statement of Eisenmann and her co-workers[6] that phosphate readily enters the red blood cells within a period of four hours at 37 5 C The present investigations are free from any criticism that the entrance of the marked phosphate was due to the increased phosphate concentration of the plasma Based as they are on direct determinations of P^{32} in both plasma and washed red cells they show that the red blood cell is permeable to the phosphate ion It is unfortunate that longer incubation studies were precluded by the physiologic changes in the red blood cell which culminated in its destruction by hemolysis In the text it was indicated that incubation studies had been carried on beyond four hours at 37 5 C Most of the data so obtained were vitiated by the hemolysis of the red cell It is worth while mentioning that at twenty three hours the intracellular distribution of phosphorus compounds in the remaining cells did not differ markedly from those obtained at four hours The slight increas in the plasma concentration of all the phosphorus derivatives is presumably due to slight hemolysis of the red blood cells

The data indicate that while over 50 per cent of the added P^{32} entered the red blood cell at 37 5 C in four hours less than 15 per cent was found in the acid soluble organic fraction the remainder having been present in the inorganic fraction At this time the specific radioactivity of the red blood cell inorganic fraction was equal to or greater than the specific activity of the plasma inorganic fraction Therefore penetration and retention of phosphate in the red blood cell under these experimental conditions cannot be entirely accounted for by the formation of organic complexes

It is well known that when whole blood is incubated at 37 5 C the blood glucose rapidly falls while when plasma or serum are so incubated the fall of glucose is very slow It is possible that the transfer of phosphate from the inorganic to the organic acid soluble form may be due to phosphorylation which precedes the utilization of glucose by cells This point is being further investigated

crease of hematocrit was found and cell destruction as indicated by marked hemolysis was present Concomitant with this cell destruction marked increases in the plasma content of all the phosphorus fractions was found For this reason data beyond an incubation period of four hours have been excluded from this communication

Phosphate Exchange between Plasma and Erythrocyte

The data are presented in table 2 It may be seen that between 91 and 105 per cent of the added P[3] was recovered or determined as present in the plasma and red cells These figures represent the limits of accuracy of the methods employed [5]

TABLE 2 —*Phosphate Excl ange betueen Plasma and Erythrocytes*

E p No	Ti ue	Temp	Initial (5 m nutes)				After 4 ho incub t n		
			P³² added† C P S per 100 ml	Total P % of added P ‡	I orga c P % of added P	Total ac d s l ble P % of add d P³²	T tal P‡ % of added P³²	I o g n P % of dded P	Total a d sol uble P % f added P³²
		C							
1	Pl m	37 5	9 6 0	—	—	—	33 (4 0)	33 9 (13 6)	33 7 (12 5)
	Red blood ll	37 5		—	—	—	57 2 (? 6)	43 6 (12 8)	57 4 (3 9)
2	Pl ma	37 5	11 640	—	—	—	3 6 (4 1)	3 9 (15 6)	36 0 (14 4)
	R d blo d ll	37 5		—	—	—	51 6 (1)	46 0 (16 7)	5 2 (3)
3	Pl m	37 5	8 961	87 1 (13 8)	—	—	34 1 (4 1)	33 8 (1 5)	33 1 (10 7)
	R d bl d ll	3 5		3 6 (0 14)	—	—	5 7 (6)	49 8 (14 2)	7 8 (3 8)
4	Pl sma	7	940	92 0 (18 5)	—	—	101 0 (18 7)	97 7 (81 4)	101 5 (78 1)
	R d bl od ll			6 (0 27)	—	—	2 9 (0 1)	2 6 (0 76)	3 2 (0 1)

All lu calc lated on wh l blood d t but on data
† C P S —c ts pe se o d pe 100 ml whole blood
‡ F ᵍu e in p nth es a e spec fi t t l l t d $\frac{\text{% f dd d t ty p 100 ml}}{\text{mg f P mpou d pe 100 ml}}$

In two experiments the per cent of the added P[3] in the total phosphorus and specific activities were determined for plasma and red cells five minutes after the addition of the P[3] At this time 3 6 and 6 7 per cent were found in the red blood cells

After incubation at 37 5 C for four hours about one third of the added radio phosphorus was detected in the plasma In each instance in the plasma there had been no turnover between the inorganic phosphate and the residual acid soluble organic or other organic forms When blood was kept at 7 C for four hours all of the added P[3] also remained in the plasma in the inorganic fraction in which form it had been added

At 37 5 C the exchange of phosphate between plasma and red blood cells was rapid from 51 to 57 per cent of the added radioactivity being detected in the washed red cells at four hours

PART VII

GENERAL PRACTICE

Additional evidence that both the penetration of the red blood cell by phosphate and the turnover from inorganic to acid soluble organic phosphorus compounds may be functions of cell metabolism is given by a comparison of the data obtained at 37 5 C with that obtained at 7 C In the latter case no significant exchange of labeled phosphate between red blood cells and plasma occurred and the small amount of P^{3} discovered in the red blood cell was confined entirely to the in organic fraction

This experience gives rise to the hope that in vivo studies of phosphate exchange in man can be undertaken using the red blood cell as a tissue cell model If this is so it will permit investigations of intermediate phosphate metabolism in man where tissues such as the liver are not readily available

Summary

1 Phosphate exchange in red cells and plasma was studied in vitro using P^{32} in the form of sodium phosphate as a tracer

2 No phosphate was added other than the isotopic preparation which was of high specific activity

3 Inorganic phosphate exchanged freely between the plasma and the erythro cytes at 37 5 C in a period of four hours Minimal transfer occurred at 7 C

4 Most of the added P^{3} which passed into the erythrocytes during this time remained in the inorganic fraction less than 15 per cent being found in the organic acid soluble fraction

5 The specific activity of the inorganic phosphate of the erythrocytes was equal to or greater than that obtaining for the inorganic phosphate of the plasma at the end of the four hour incubation period at 37 5 C

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PART VII

GENERAL PRACTICE

THE OCCUPATIONAL INCIDENCE OF PEPTIC ULCER IN DENMARK

By GUNNAR ALSTED M D

THE EXACT determination of the incidence of peptic ulcer in a population is most difficult Reporting of the disease is never required and as of itself it is not generally fatal no information can be obtained from the mortality statistics Consequently other methods of investigation have to be employed A study of the incidence of ulcers and scars in postmortem material leads to some information on the subject However the results are most variable giving an ulcer rate from 1–2 per cent up to 20 per cent depending among other factors on the carefulness of the investigators and the completeness of the postmortem examination In general the incidence is stated as 4–5 per cent but this method of course yields no information about the actual incidence of peptic ulcer in the entire population

Another way of obtaining information is the clinical method based on the ulcer rate found among hospital patients Clinical data must however be regarded as even more unreliable than that obtained from postmortem studies because diagnostic criteria clinical investigation and the character of the patient material have varied from period to period and from place to place to such an extent that comparison can hardly be made from one hospital department to another and not at all from one period to another As examples of newer statistics of this nature I can mention that Alvarez[1] in 1929 in the Mayo Clinic found radiologic evidence of ulcer in 3 3 per cent of all his patients while Steigman[11] found ulcer in only 0 9 per cent of 68 000 patients admitted to Cook County Hospital Chicago in 1934

The less selected and the bigger the groups of individuals examined the lower of course will the ulcer rate turn out to be and in an investigation such as the following which comprises the entire population of Denmark (3 706 349) the incidence should be still less Actually the mean ulcer rate here is 11 2 per 10 000 inhabitants although in certain age groups and occupations it is many times higher

However although the absolute ulcer rate is important of much more interest from an etiologic point of view are differences in frequency The existence of fluctuations in the incidence of peptic ulcer due to geographic distribution and occupational hazard has long been known

It is a well established fact that the ulcer rate in certain countries is extremely low e g in Ethiopia as stated by Bergsma [2] According to MacCarrison [8] ulcer is practically unknown in Northern India while it occurs frequently in Southern India a fact which may be explained by the different dietary habits of the population Likewise dietary factors may be a predominating cause of the high ulcer rate in the Chinese and the low rate in Indian coolies reported from Java by Kouwenaar [7] On the other hand Jelinek[6] found no more than 20 ulcer cases among 51 000 patients in South China These are but a few examples from the literature on this subject from which it appears that the ulcer rate is higher in civilized communities and urban areas than in primitive races and rural districts

From the Frederiksberg Hospital Copenhagen Denmark

Investigations of occupational factors influencing the ulcer rate are comparatively few There seems to be no single occupation which especially predisposes to ulcer However Eusterman and Balfour[4] state in their book that the disease is common in individuals living under great nervous strain and having heavy responsibilities but admit that the proof is still lacking Nevertheless on reviewing the recent reports on ulcer and occupation one is impressed by the fact that an elevated ulcer rate seems to prevail in certain occupations Thus 15 per cent of Church and Hinton s[3] 671 ulcer cases from Bellevue Hospital in New York occurred in chauffeurs automobile mechanics and car drivers Wiebel and Kunstreich [13] at the University Clinic in Marburg found the highest ulcer rate 9 1 per cent in motor drivers, farm laborers and farmers the rate otherwise being 7-8 per cent Schellong[10] found the ulcer rate in builders workmen three to four times as high as in butchers tailors and cobblers Weidinger [12] in Munich found the highest incidence in drivers laborers and artisans the lowest in farmers In Sweden Ihre and Muller[5] found a remarkably high ulcer rate among commercial travellers tram and railway employees seamen and to a certain extent drivers Finally Schancke [9] in a recent extensive study from Northern Norway found the ulcer rate considerably higher in fishermen than in all the other occupational groups taken together Apart from these reports little is to be found in the literature dealing with occupational factors influencing the development of peptic ulcer

The following communication is part of an extensive study carried out by the author working through the Danish National Health Service with the object of elucidating the manifestations of peptic ulcer in the Danish population as well as its geographic and occupational incidence The investigation took place during the German occupation of Denmark This fact and subsequent events have delayed its publication in English The investigation covered the whole country and was carried out by requesting all doctors in Denmark both general practitioners and specialists and all hospitals to notify the local public health authorities of the number of ulcer patients seen or treated by them in October 1940 The notification was part of a special questionnaire allowing for information about the patient and the character of the ulcer All reports were finally collected by the author and statistically analyzed

As only about 75 per cent of the doctors filled and returned the forms no information about the absolute ulcer rate could be obtained but since the returns showed that the noncooperative doctors were equally distributed over the country I believe that the results convey a satisfactory impression of the distribution of peptic ulcer in the various groups of the population In total 4159 ulcer cases were reported 3113 male and 1046 female giving a male to female ratio of approximately 3 to 1 In a population of 3 706 349 out of which 1 824 289 are men and 1 882 060 women this gives a mean overall ulcer rate of 11 2 per 10 000 of the population 17 1 per 10 000 men and 5 6 per 10 000 women

Before turning to the incidence of ulcer in the various occupational groups it would be natural to subdivide the population in groups according to its domicile a subdivision which is closely related to the occupational since the place of domicile in Denmark is more or less determined by the occupation of the individual

In this way the population can be subdivided into the following groups (1) the population of Copenhagen, with suburbs (greater Copenhagen) (2) the provincial towns (3) urban agglomerations and (4) rural areas This subdivision and the ulcer rate within each of these four principal groups are shown in table 1

It is here immediately obvious that while the ulcer rate is almost uniform in Copenhagen, the provincial towns and the urban agglomerations i e about 12–13 per 10 000 it is essentially lower in the rural areas i e , 8 7 per 10 000 This low ulcer rate in the rural population is caused by a low male ulcer rate i e 12 3 per 10 000 against about 20 per 10 000 in the remaining male population The female ulcer rate in the rural population is 4 8 per 10 000 against 6 per 10 000 in the remaining female population This gives in the rural districts a male ulcer preponderance of 2 6 to 1 against 3 6 to 1 in the remaining population

As we are dealing with groups comprising about 1 000 000 individuals each, the differences observed must be regarded as true and it seems to be a fact that the incidence of peptic ulcer is less in the rural population of Denmark than in the urban

TABLE 1 —*Ulcer Rate and Domicile*

	Census Nov 5 1935			Ulcer cases per 10 000 Oct. 1940		
	Males	Females	Total	Males	Females	Total
Greater Copenhagen	384 804	458 364	843 168	19 6	5 6	12 0
Provincial towns	410 112	454 888	865 000	21 0	5 9	13 1
Urban groups	290 055	299 896	589 951	20 3	6 8	13 5
Rural areas	739 318	668 912	1 408 230	12 3	4 8	8 [illegible]
Whole country	1 824 289	1 882 060	3 [illegible]06 349	1[illegible] 1	5 6	11 2

population This applies in particular to men in rural districts who evidently are less afflicted by peptic ulcer than men in other parts of the country

Peptic ulcer shows a characteristic age distribution which is more pronounced in men than in women and it is therefore of interest to investigate the age distribution within each of the four principal groups of the population In table 2 is shown the ulcer rate per 10 000 subdivided in age groups of 10 years

From table 2 it appears that while the male ulcer rate in Copenhagen the provincial towns and the urban agglomerations rises sharply with age with a maximum in the group between the ages of 40–59 and later declining abruptly the male ulcer rate in rural areas rises much less thus proving clearly that the low incidence among rural males is caused by a low incidence among men between 40 and 59 In women the age distribution curve runs an almost parallel course in the four groups in question

Since the study is based on large numbers of cases the difference in the incidence of ulcer in different localities as demonstrated in this paper is probably valid However it might be objected that peptic ulcer is less frequently diagnosed in rural areas than in urban communities However access to advanced clinical examination and x ray facilities are in a country like Denmark about equal in the

Investigations of occupational factors influencing the ulcer rate are comparatively few There seems to be no single occupation which especially predisposes to ulcer However Eusterman and Balfour[4] state in their book that the disease is common in individuals living under great nervous strain and having heavy responsibilities but admit that the proof is still lacking Nevertheless on reviewing the recent reports on ulcer and occupation one is impressed by the fact that an elevated ulcer rate seems to prevail in certain occupations Thus 15 per cent of Church and Hinton s[3] 671 ulcer cases from Bellevue Hospital in New York occurred in chauffeurs automobile mechanics and car drivers Wiebel and Kunstreich [13] at the University Clinic in Marburg found the highest ulcer rate 9 1 per cent in motor drivers farm laborers and farmers the rate otherwise being 7–8 per cent Schellong[10] found the ulcer rate in builders workmen three to four times as high as in butchers tailors and cobblers Weidinger,[1] in Munich found the highest incidence in drivers laborers and artisans the lowest in farmers In Sweden Ihre and Muller[5] found a remarkably high ulcer rate among commercial travellers tram and railway employees seamen and to a certain extent drivers Finally Schancke [9] in a recent extensive study from Northern Norway found the ulcer rate considerably higher in fishermen than in all the other occupational groups taken together Apart from these reports little is to be found in the literature dealing with occupational factors influencing the development of peptic ulcer

The following communication is part of an extensive study carried out by the author working through the Danish National Health Service with the object of elucidating the manifestations of peptic ulcer in the Danish population as well as its geographic and occupational incidence The investigation took place during the German occupation of Denmark This fact and subsequent events have delayed its publication in English The investigation covered the whole country and was carried out by requesting all doctors in Denmark both general practitioners and specialists and all hospitals to notify the local public health authorities of the number of ulcer patients seen or treated by them in October 1940 The notification was part of a special questionnaire allowing for information about the patient and the character of the ulcer All reports were finally collected by the author and statistically analyzed

As only about 75 per cent of the doctors filled and returned the forms no information about the absolute ulcer rate could be obtained but since the returns showed that the noncooperative doctors were equally distributed over the country I believe that the results convey a satisfactory impression of the distribution of peptic ulcer in the various groups of the population In total 4159 ulcer cases were reported 3113 male and 1046 female giving a male to female ratio of approximately 3 to 1 In a population of 3 706 349 out of which 1 824 289 are men and 1 882 060 women this gives a mean overall ulcer rate of 11 2 per 10 000 of the population 17 1 per 10 000 men and 5 6 per 10 000 women

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[illegible] occupational groups [illegible] will be [illegible] All of these [illegible] results of this investigation [illegible] principal [illegible] number of ulcer cases per [illegible] age groups. Group 6 [illegible] is most heterogeneous [illegible] or without stated occupation. It is [illegible] claim the chief [illegible] of limited value. The two middle age groups [illegible] in very young persons [illegible] was found to be extremely rare in [illegible] comparatively small [illegible] men included in the last age group was [illegible] Comparable [illegible] standard error of the [illegible] groups [illegible] which shows that only the differences [illegible] in the two middle groups are [illegible] statistically valid. The results confirm the low ulcer rate [illegible]

Table 3.—Ulcer Cases per [illegible]

Occupational groups (number of men)	[illegible]	[illegible]	[illegible]	[illegible]
1) Agriculture, Forestry and Fishing [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2) Handicraft and industry [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3) Trade and commerce [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4) Traffic and transport [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5) Office administration and liberal professions [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6) Men without or with unstated occupation [illegible]	[illegible]	[illegible]	[illegible]	[illegible]

the rural population. In the group comprising agriculture, forestry and fishing, the difference being most pronounced in men from 45–64 years of age. The other principal occupational groups show a considerable increase occurring with advancing age.

The differences between the remaining principal occupational groups were less pronounced. There was, however, a clear distinction between the groups comprising trade and commerce, office administration and the liberal professions on one hand, and the groups comprising handicraft, industry and traffic and transport on the other hand, the ulcer rate in the important age groups being higher in the former occupations than in the latter, however. The group comprising traffic and transport exhibits an extremely high ulcer rate among men over 65. This is a small group of only 2500 individuals and the high rate must therefore be regarded with reservation. However, it may be of some significance, particularly in view of other reports on the occupations

country and in the city Furthermore the changes observed were associated with only certain age groups in men while the remaining male age groups and all groups of females show the same ulcer rate throughout the country

No final explanation of the difference in the ulcer rate can be obtained from this material However between rural and urban populations as the difference is observed only in men it may be suggested that it is occupationally conditioned Thus farming being the most common occupation in rural areas evidently does not predispose to development of ulcer in the age groups where this disease is otherwise most frequent The uniform work chiefly of physical nature and the regular life habits may be of importance Nutritional factors seem to play no role, as can be deduced from the fact that the female ulcer rate is the same all over the

TABLE 2 —*Age Distribution of Peptic Ulcer*

	Cases per 10 000						
	0–19	20–29	30–39	40 49	50 59	60–69	70–
Greater Copenhagen							
Males	0 6	8 0	24 5	46 4	43 4	29 1	19 4
Females	0 5	3 3	5 3	11 5	10 4	10 1	6 3
Total	0 5	5 4	14 0	27 4	25 0	18 2	11 0
Provincial towns							
Males	1 2	12 4	37 6	49 2	42 0	28 0	12 6
Females	0 5	2 1	8 0	11 3	15 9	12 6	6 7
Total	0 8	6 8	21 9	29 4	28 4	19 7	9 2
Urban groups							
Males	1 1	10 8	37 1	42 9	43 5	40 3	13 6
Females	0 8	2 9	8 8	15 4	12 5	15 7	11 7
Total	0 9	7 4	22 6	28 8	27 8	27 6	12 6
Rural areas							
Males	0 8	7 3	22 7	28 5	28 2	24 5	10 9
Females	0 4	3 7	7 1	8 5	12 5	9 3	8 2
Total	0 6	5 7	15 3	18 6	20 6	17 3	9 6

country There was by the way no reason to expect any variation in the diet within the four principal groups of the population The population in the rural areas and in the urban agglomerations at least lives more or less on the same diet In the official statistics applied here it seemed to make no statistical difference whether a community was classified as belonging to one or the other of the same two groups

Several difficulties are encountered when approaching the question of the occupational incidence of peptic ulcer in an extensive population First the investigation must necessarily deal with men alone the statement of occupation being in the case of most of the women far too uncertain to be of any use However this fact is relatively unimportant since peptic ulcer is 3–4 times as frequent in men as in women Secondly the subdivision of the material into occupational groups results in some groups so small that the differences observed may not be statistically valid Thus no absolute proof can be provided only more or less well founded sup-

positions may be obtained Thirdly if one considers only those occupational groups of sufficient size to make the results statistically valid information will be lacking concerning certain details which later may prove to be important All of these considerations must be kept in mind when judging the results of this investigation

In table 3 are presented the number of ulcer cases per 10 000 men in six principal occupational groups each of which is subdivided into four age groups Group 6 comprises men either without or with stated occupation It is most heterogenous and consequently of limited value The two middle age groups claim the chief interest since peptic ulcer was found to be extremely rare in very young persons and the number of men included in the last age group was comparatively small This can readily be seen when the standard error of the different groups is calculated and clearly shows that only the differences observed in the two middle groups are statistically valid The results confirm the low ulcer rate previously mentioned in

TABLE 3 —Ulcer Cases per 10 000 Men in 6 Principal Occupational Groups

Group and number of individuals in each	0- 4	25-44	45-64	65	Total
1) Agriculture Forestry and Fishing 438 245	3 0 ± 0 4	19 0 ± 1 1	26 6 ± 1 6	25 0 ± 3 3	15 6 ± 0 6
2) Handicraft and industry 349 438	6 5 ± 1 0	3 5 ± 1 5	46 9 ± 2 2	35 2 ± 4 8	33 8 ± 1 0
3) Trade and commerce 106 4 3	8 8 ± 2 0	40 0 ± 2 9	62 0 ± 4 5	49 ± 10 1	40 1 ± 1 9
4) Traffic and transport 94 964	6 4 ± 2 3	25 4 ± 2 1	43 1 ± 3 8	115 0 ± 21 2	30 ~ ± 1 8
5) Office administration and liberal professions 105 695	5 ± 1 4	39 4 ± 2 9	60 6 ± 4 8	65 1 ± 12 5	36 ± 1 9
6) Men without or without stated occupation 1 6 00	5 ± 2	32 9 ± 5 1	20 4 ± 3 0	4 9 ± 0 8	11 2 ± 1 0

the rural population In the group comprising agriculture forestry and fishing the mean ulcer rate was lower than in the remaining occupations the difference being most pronounced in men from 45 64 years of age The other principal occupational group show a considerable increase occurring with advancing age

The differences between the remaining principal occupational groups were less pronounced There was however a clear distinction between the groups compris ing trade and commerce office administration and the liberal professions on one hand and the groups comprising handicraft industry and traffic and transport on the other hand the ulcer rate in the important age groups being higher in the former occupations than in the latter however The group comprising traffic and transport exhibits an extremely high ulcer rate among men over 65 This is a small group of only 2 500 individuals and the high rate must therefore be regarded with reservation However it may be of some significance particularly in view of other reports on the occupations

The difference in the ulcer rates observed in the middle age groups of the princi pal occupational groups must be regarded as true but apart from the low rate in the agricultural group, no certain information is obtained as to which occupation in particular differs from the average To investigate this question more thor

TABLE 4 —*Ulcer Cases per 10 000 Males in Various Occupations and Professions*

	Number	25-44	45-64
1			
Landowner farmer	91 567	21 9	27 3
Small holder and the like	91 912	16 4	23 3
Fisherman	14 072	35 5	27 7
Ag icultural laborer and workingman	231 418	14 5	30 0
2			
Laborer in food and nutrition industry	34 658	32 8	55 0
Laborer in wood and furniture industry	44 315	28 9	51 5
Laborer in iron and hardware industry	59 612	29 8	40 8
Earth and concrete laborer bricklayer	25 563	25 6	58 2
Barber hair cutter	5 533	38 5	55 2
All laborers and tradesmen	248 259	44 2	66 1
3			
Bank manager stock broker wholesale dealer	6 664	62 2	78 0
Retailer and small trader	37 585	40 8	57 8
Executive and assistant in restaurant hotel and pension	11 545	32 2	55 6
4			
Superior employee in railway tramway and postal services	5 922	36 7	26 4
Subordinate employee in same services	20 254	19 3	62 5 (241 8)
Coachmaster freight driver	10 392	32 4	37 4
Motor driver coachman messenger	28 666	32 4	50 0
Ship s officer seaman stoker	8 608	36 7	33 9
5			
Executive office employee	24 011	42 7	70 9
Subordinate office employee	25 658	28 9	37 8
Minor civil servant non comissioned officer	13 470	52 5	58 2
Lawyer	2 120	27 7	36 7
Teacher	11 518	33 3	38 7
Clergyman	1 683	26 1	37 6
Architect journalist artist	9 607	44 1	60 9
Physician Surgeon	3 204	55 2	116 5

oughly it would be necessary to study each single occupation which would in volve dealing with small numbers of cases Therefore in the following part of the investigation only occupations or occupational groups of a certain numerical size are included except for a few occupational groups which though numerically small are distinguished by a special homogeneity or in which the conditions for

other reasons are particularly well illustrated or clear cut Furthermore as a rule only the age groups from 25 to 64 years are included the younger and older age groups being for reasons already stated of less interest All results have been subjected to standard error computation but because of the comparatively small groups the standard error in all groups is so large that none of the differences observed is larger than three times the standard error The validity of such statistical analyses may be questioned

Table 4 gives the number of ulcer cases per 10 000 men in the various occupations observed and in a few collective groups The ulcer rate is somewhat higher in large scale farmers than in small holders The difference observed may not be large it is however probably valid because of the heterogeneity and the numerical size of these occupations It is certainly not caused by a different age distribution in the two occupations as it is constant in the two important age groups On the other hand the large but extremely heterogenous group comprising farm hands and agricultural laborers has a very low ulcer rate in the younger individuals and a high rate in the older persons

Within the handicraft and industry group a few collective groups have been studied These have a higher ulcer rate than that observed in the agricultural groups in both age groups in question The lowest frequency is observed in laborers in iron and the hardware industry while the highest rate is found in concrete and earthwork laborers and bricklayers This applies in particular to the older age group but the difference is hardly big enough to allow safe statistical analysis

The group of barbers and hair cutters though numerically small has been studied because of its homogeneity A collective group of more than 248 000 men comprising all laborers artisans and tradesmen has an ulcer rate of 44 2 and 66 1 per 10 000 respectively in the two important age groups in this form of employment This rate was higher than was observed in any other single large occupation presented and very much higher than in any agricultural occupation

When the main group trade and commerce are broken down the single occupational groups are small It is striking that a collective group of banking managers stock brokers and wholesale dealers i e persons with a high average annual income and a consequent high standard of living has a very elevated ulcer rate However this does not seem to be caused by eating and drinking alone as the group of employees in the restaurant and hotel trade has an essentially lower ulcer rate However both groups mentioned are small and the percentages must be conservatively evaluated

In the principal occupational group comprising all men employed in traffic and transport the subdivisional groups are again small Most conspicuous is the group of subordinate personnel in the railway tramway and postal services a group comprising more than 20 000 men This group has an incidence of ulcer of 19 3 per 10 000 in the age group from 25 to 44 years and the rate rises abruptly to 62 5 per 10 000 in the following age group from 45 to 64 years Men over 65 in this group have the excessively high ulcer rate of 241 8 per 10 000 The last age group comprises 455 men only but even so the abrupt rise of the ulcer rate with advancing age may signify that men employed in occupations within traffic and transport

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easy access to x rays It is likewise to be supposed that any doctor suffering from ulcer at the time of this inquiry has been interested in this disease to the extent that he has reported his own case

But even with these reservations there is no doubt that the medical profession has a markedly elevated ulcer rate higher or at least as high as any other occupation or profession This fact cannot be satisfactorily explained from the material in hand It may however be suggested that the combination of irregular working hours as observed among the driving personnel with the responsibility of the work as seen in a few other occupations is a factor at least contributive to the high ulcer rate among doctors

As a concluding observation in cases where I have been able to secure reliable information about the average annual income I have tried to correlate it with the ulcer rate in the various occupations Although the rate seems to be highest in the occupations and professions with the highest incomes no certain relation can be demonstrated It seems to be the nature of the occupation rather than the size of the income which determines the frequency of peptic ulcer

Summary and Conclusions

The results of a census of ulcer cases made through the Danish National Health Service in October 1940 are analyzed On the basis of the 4159 cases reported the occupational incidence of the disease is discussed It appears that whereas the incidence is more or less the same in Copenhagen with suburbs (greater Copenhagen) provincial towns and urban agglomerations i e about 12–13 cases per 10 000 inhabitants it is essentially lower in the rural areas i e 8 7 per 10 000 It furthermore appears that this low ulcer rate among the rural population is due especially to the low incidence of the disease among males i e 12 3 cases per 10 000 males as against about 20 per 10 000 among the rest of the population The female ulcer incidence among the rural population is 4 8 per 10 000 females as against 6 per 10 000 among the rest of the population The male preponderance of the disease in rural areas is therefore only 2 6 to 1 as against 3 6 to 1 among the remaining population

It further appears that the low ulcer rate among males in the rural areas is due especially to the low incidence among the age group 40–59 years For females the age distribution curve is almost the same in all the four principal groups of the population From this last it must be supposed that dietary conditions have nothing to do with the low ulcer incidence in the rural areas

Statistical analysis of the occupational distribution of ulcer shows that the highest incidence is found among doctors and among men in leading positions in commerce and trade or holding executive office positions Employees in traffic and transport show a markedly increasing ulcer frequency with advancing age A high ulcer incidence is found also among workingmen and artisans Any certain difference in that respect between the different trades and occupations cannot be demonstrated however Retailers and hotel and restaurant employees have a somewhat lower ulcer incidence the liberal professions and subordinate office employees a

become increasingly predisposed to ulcer as they grow older These men are chiefly employed in vehicular driving having extremely irregular working and eating hours Therefore it may be suggested that such working hazards and conditions in particular predispose to peptic ulcer This conclusion is in perfect accordance with the clinical experience both of the present author and others Against it may be argued and quite rightly that men in similar occupations such as car and freight drivers coachmen and motor drivers do not show an ulcer rate essentially higher than laborers and tradesmen The same low incidence applies to all sailing personnel, both officers, seamen and stokers However the last fact may be offered as further proof of the insignificance of dietary factors for the development of peptic ulcer Anybody familiar with sailors fare will surely agree

The last group studied comprises men employed in office work and administration and in the liberal professions This is a group difficult to evaluate since the big subgroups are extremely heterogenous and the homogenous subgroups are very small However most interesting is the widely differing ulcer rates observed in two comparable groups the executive and the subordinate office personnel the ulcer rate being twice as high in the former as in the latter It might be suggested that the ulcer rate at least to a certain extent depends on the responsibility of the occupation and this is in accordance with the results previously mentioned On the other hand the group of minor civil servants and subordinate military personnel shows a comparatively high ulcer rate in both age groups represented i e 52 2 and 58 2 per 10 000 respectively

This group by the way is the only one where the author has been able to control the absolute numerical value of the material I had the opportunity of looking over the case records of all policemen employed in the Metropolitan Police Force of Copenhagen Among about 2000 employees all between 25 and 65 I found 13 cases of peptic ulcer occurring during October 1940 This corresponds to an ulcer rate of 65 per 10 000 and is in good accordance with the incidence of ulcer observed in the group of which it is a part This confirms the impression of the general validity of the ulcer rates observed in this investigation

Among the liberal professions the comparatively homogenous groups of lawyers teachers and clergymen have a rather uniform ulcer rate which is somewhat below the average for this principal occupational group as a whole whereas the extremely heterogenous groups of architects journalists and artists has a somewhat higher rate

Finally mention should be made of the medical profession It is at once evident that as to the ulcer rate (as in many other aspects of life) doctors seem to have a peculiar position their ulcer rate being 55 2 and 116 5 per 10 000 in the two main age groups i e considerably higher than any other ulcer rate observed in the corresponding age groups in other occupations and professions However certain reservations are necessary First both the diagnosis and the notification of ulcers are better and more safe among doctors than in the remaining population as most likely any doctor suffering from dyspeptic symptoms will try to make a certain diagnosis this can hardly be said of other occupational groups Furthermore doctors undoubtedly have better facilities for special examination in particular

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very much lower The lowest incidence by far is found among the rural population, and there it is undoubtedly lower among the small holders than among the big farmers

The causes of the different ulcer incidence in the different occupations can only be surmised They do not with certainty seem to have any relation either to average income or to food conditions Among groups of the population where the food must be supposed to be poor, the frequency of ulcer seems if anything to be less than among groups where the conditions in that respect must be presumed to be better At the same time the incidence is not particularly high in the occupations where it is generally recognized that the consumption of food and drink is abundant nor in those occupations where the quality of the food owing to the circumstances such as in ships at sea cannot always be ideal

On the other hand there seems to be a certain relation between the nature of the occupation and the frequency of ulcer Thus the rural population whose habits of life must on the whole be supposed to be regular and whose work may probably be said to demand greater physical than intellectual exertion has the absolutely lowest ulcer incidence Continued irregular life habits with irregular hours for meals and rest must be supposed to increase the liability to ulcers as we see it from the figures for subordinate tram and railway employees Finally the highest ulcer rate is found among men whose office and occupation involve personal responsibility and who are often called upon to make decisions and take measures of greater or lesser import and magnitude

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ONE HUNDRED CASES OF DIABETIC COMA (UNCONSCIOUSNESS)

By W Richard Ohler M D

THE PURPOSE of this communication is to report 100 consecutive cases of diabetic coma from the Boston City Hospital and also to discuss certain matters pertaining to the general problem of diabetic coma

Definition of Diabetic Coma

Unfortunately, a certain amount of confusion exists because some authors diagnose coma on the basis of blood CO_2 content while others classify patients on the basis of the clinical picture without regard to chemical data For example Joslin[2] uses a blood CO_2 of 20 volumes per cent as an arbitrary dividing line between precoma and coma On the other hand Rabinowitch and his co workers subdivide cases in four groups (1) drowsy (2) semiconscious (3) responding to pain (4) completely unconscious In other words despite the fact that the word coma means a state of unconsciousness when it is applied to diabetes and particularly to statistics relating to diabetes some authors intend the literal definition of the word while others group under the general classification of coma both conscious and unconscious patients depending entirely upon certain chemical findings The current use of the term *diabetic coma* therefore may be quite misleading since some authors use it to denote severe acidosis while others use it to denote unconsciousness of varying degree

A brief review of the essential data from two patients recently treated in this clinic will serve to emphasize the points outlined above

Case 1

E M a 46 year old woman with a four year history of diabetes well controlled by insulin entered the hospital in deep coma with typical clinical signs of diabetic acidosis and absent corneal reflexes Stupor and inability to recognize members of the family was noted fourteen hours before her admission

On admission the blood sugar was 650 mg and the CO_2 combining power was less than 10 volumes per cent At the end of twelve hours in the hospital the patient was responsive and taking fluids by mouth and at the end of twenty four hours she was alert and hungry During the first twelve hours she received 800 units of insulin and approximately 8 000 cc of fluid The composition and route of administration of the fluid was as follows by the intravenous route 4 000 cc normal saline solution 5 per cent dextrose in saline 1 500 cc M/6 Lactate solution 500 cc by hypodermoclysis saline solution 800 cc by stomach tube milk fruit juice water 1 200 cc

At this hospital this patient is classified as a case of diabetic coma with recovery

Case 2

L B a 60 year old male diabetic previously on insulin entered the hospital conscious rational able to give an adequate history On admission the blood sugar was 610 mg the blood CO_2 less than 10 volumes per cent and the N P N 74 mg During the first twelve hours he received 800 units insulin 6 000 cc saline solution 4 200 cc 5 per cent glucose in saline and 500 cc lactate solution all by the intravenous route At the end of this period his blood CO_2 was 48 per cent his blood sugar 160 mg and his N P N 60

He was discharged as a case of severe diabetic acidosis with recovery

From the Thorndike Memorial Laboratory Boston City Hospital Boston Mass

very much lower The lowest incidence by far is found among the rural population and there it is undoubtedly lower among the small holders than among the big farmers

The causes of the different ulcer incidence in the different occupations can only be surmised They do not with certainty seem to have any relation either to average income or to food conditions Among groups of the population where the food must be supposed to be poor the frequency of ulcer seems if anything to be less than among groups where the conditions in that respect must be presumed to be better At the same time the incidence is not particularly high in the occupations where it is generally recognized that the consumption of food and drink is abundant nor in those occupations where the quality of the food, owing to the circumstances such as in ships at sea cannot always be ideal

On the other hand there seems to be a certain relation between the nature of the occupation and the frequency of ulcer Thus the rural population whose habits of life must on the whole be supposed to be regular and whose work may probably be said to demand greater physical than intellectual exertion has the absolutely lowest ulcer incidence Continued irregular life habits with irregular hours for meals and rest must be supposed to increase the liability to ulcers as we see it from the figures for subordinate tram and railway employees Finally the highest ulcer rate is found among men whose office and occupation involve personal responsibility and who are often called upon to make decisions and take measures of greater or lesser import and magnitude

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1 *Degree and duration of unconsciousness* As previously stated, we have no accurate data on either the degree or the duration of unconsciousness

2 *Age* The average age of those who lived was 32 years the youngest patient was 10 years and the oldest 73 years The average age of those who died was 54 years the youngest, 14 years and the oldest 72 years The influence of age on mortality becomes more apparent when it is pointed out that among those who died there were only two patients below 20 years of age and of the remaining twenty one patients all were over 30 years of age

3 *Complications* Here the difference is striking Of those who lived 22 per cent had serious complications as opposed to 91 per cent with serious complications among those who died

4 *Blood Pressure* Of the fatal cases 40 per cent had blood pressure below 100 systolic and four patients had no measurable pressure In sixty six patients who recovered whose initial blood pressure was recorded 22 per cent had blood pressures below 100 systolic and four had no measurable blood pressure

5 *Blood* CO_2 In forty two living cases in whom the initial blood CO_2 is recorded the average was 12 volumes per cent with the lowest reading 4, and the highest reading 20 In fifteen fatal cases in whom the initial blood CO_2 was recorded the average was 14 volumes per cent with the lowest reading 6, and the highest 23 It is quite apparent that in this combined group of fifty seven cases the level of the blood CO_2 has no influence on mortality

SUMMARY

From the above mentioned data it is apparent that the three conditions which definitely influence prognosis in our series were age the presence or absence of serious complications and the blood pressure As noted above we have no accurate data on the duration of unconsciousness prior to the introduction of active treatment

CONCLUSIONS

1 In an unselected series of one hundred consecutive patients in diabetic acidosis with unconsciousness of undetermined duration at the time of admission the mortality was 23 per cent

2 Bearing in mind that we have no accurate data on the length of unconsciousness prior to treatment the three factors which appeared to affect mortality in our series were age the presence of absence of serious complications and the blood pressure level

3 Attention is directed to the misuse of the word coma as applied to diabetics

REFERENCES

1 JOSLIN E P ET AL The Treatment of Diabetes Mellitus Lea and Febiger 8th Ed p 410
RABINOVITCH I M ET AL Diabetic coma Ann Int Med 1403 (new Series 12) 1938-39
JOSLIN E P ET AL The Treatment of Diabetes Mellitus Lea and Febiger 8th Ed p 433

We recognize that the complications of severe acidosis without unconsciousness may be just as severe as it is in the unconscious patient nevertheless it is our opinion that the problems of treatment are much more difficult in the unconscious patient and we are equally certain that the mortality is much lower in the conscious patient As a matter of fact throughout the various separate services which comprise the medical section of this hospital no serious problem is encountered in the management of the conscious diabetic regardless of the severity of the acidosis as measured by chemical data It can be stated at this time that in the group here presented, all of the cases were unconscious in that they were unable to respond Unfortunately however we have no data regarding degrees of unconsciousness as enumerated by Rabinowitch

The Natural Course of Events in Diabetic Acidosis

The symptoms of diabetic acidosis may be enumerated as follows (a) Increase in diabetic symptoms especially polyuria and polydipsia (b) Drowsiness and restlessness (c) The onset of abdominal symptoms such as nausea vomiting and abdominal tenderness These symptoms may give rise to the erroneous diagnosis of surgical condition in the abdomen (d) The onset of Kussmaul breathing (e) Increased drowsiness (f) Unconsciousness Associated with these clinical symptoms certain definite chemical changes take place the end results of which are hemoconcentration dehydration loss of base and chloride reduction in the blood CO_2 and a shift of the pH to the acid side As a result of these changes the blood pressure eventually falls and anuria develops

The only reason for calling attention to the above natural course of events is to emphasize the fact that well controlled diabetics do not present this picture or any part of it In other words when diabetics receive a sufficient amount of insulin they will not go into diabetic coma The converse is also true Given the symptoms and signs of either approaching or well established diabetic acidosis the first principle of treatment is insulin in sufficient amounts Too often this principle is lost sight of we become interested in various complications of diabetic acidosis and forget about the importance of prompt and courageous insulin therapy In this connection it is important to direct attention to the decreasing efficiency of insulin in acidosis as emphasized by Joslin [3]

Discussion of One Hundred Cases

These cases were unselected and as a group represent the type of problem encountered in a large municipal hospital

Mortality Statistics

The mortality rate for our series was 23 per cent Consideration of the fact that we were dealing with unconscious patients and consideration of published data from other clinics leads us to believe that the above mortality rate is neither good nor bad For the purposes of classification and instruction our statistical data may be broken down as follows

1 *Degree and duration of unconsciousness* As previously stated we have no accurate data on either the degree or the duration of unconsciousness

2 *Age* The average age of those who lived was 32 years the youngest patient was 10 years and the oldest 73 years The average age of those who died was 54 years the youngest 14 years and the oldest 72 years The influence of age on mortality becomes more apparent when it is pointed out that among those who died there were only two patients below 20 years of age and of the remaining twenty one patients all were over 30 years of age

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